

EFFECTS OF FREQUENCY OF COLLECTION ON QUANTITATIVE AND QUALITATIVE CHARACTERISTICS, FERTILITY AND HATCHABILITY OF CHICKEN SEMEN

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ABSTRACT: Forty adult cocks and ninety hens of Fayoumi strain were equally allotted into five groups to study the effects of semen collection frequency per week on its properties, fertility and hatchability. Ejaculate volume, sperm cell concentration and sperm number per ejaculate were determined for each cock ejaculate. Significant differences were observed for ejaculate volume ($P < 0.05$). Cocks handled for semen collection once or twice per week had larger ejaculate volumes than cocks used at higher frequencies, but no significant differences between groups were obtained for sperm concentration ($P > 0.05$). Thus there were significant differences ($p < 0.05$) for total sperm number per ejaculate between groups. However, daily sperm output (DSO) was markedly increased in cocks handled at the highest frequency (e.g., $DSO = 0.33 \times 10^9$ vs 1.08×10^9 sperm in males handled once vs seven times / week). The present results exhibit a favorable effect of high semen collection frequencies on sperm viability and a marked decline in sperm viability during the first two days following a two days resting period in cocks handled 5 times per week. Therefore, frequency of semen collection, within the ranges studied, had no adverse effect on fertilizing capacity and hatchability.

It can be concluded that Fayoumi cocks express their optimal reproductive capacity more efficiently when semen collection is undertaken at a high rather than a low frequency. Also, frequency of semen collection had no negative effects on fertility and hatchability of Fayoumi cocks.

INTRODUCTION

The interest of using the best genetic potential available from male line sires and the absence of adequate procedures to handle poultry semen over long periods are two major reasons to optimize semen collection frequency in artificially inseminated flocks. Generally, semen is collected from poultry males once or twice per week in most commercial operations, despite the absence of physiological bases to support this pattern. Previous studies showed an inverse relationship between frequency of semen collection and ejaculate volume, whereas sperm concentrations remain unaffected (McCartney *et al.*, 1958 and Cecil *et al.*, 1988). Moreover, some indications exist in the above publications that changes in the frequency of semen collection do not affect fertility and hatchability traits in artificially inseminated females (McDaniel and Sexton, 1977 and Cecil, 1982). These results indirectly contradict observations *in vitro* by Brown (1968) that increasing the frequency of semen collection significantly reduces the percentage of sperm abnormalities.

From a theoretical standpoint, the number of semen doses available per week per cock rather than the number of doses per ejaculate should be considered to quantitative the final efficacy of semen collection protocols as this provides an optimal response to determine sex ratios in breeder flocks.

The present study was conducted to determine the consequences of various semen collection frequencies (from once per week to once every day) on several quantitative characteristics of chicken semen (*i.e.*; ejaculate volume, sperm cell concentration, total sperm per ejaculate and daily sperm output), also to determine their possible effects on the viability of freshly ejaculated sperm and the relationship of frequency of collection to fertilizing capacity and hatchability of semen obtained from chicken males.

MATERIALS and METHODS

The present study was carried out at the Poultry Research Unit, Animal Production Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.

Animals and their management :

Forty adult cocks and ninety hens of Fayoumi chicken strain at twenty three weeks of age were used. The birds were randomly chosen and equally divided into five groups. Each group consisted of two replicates of four cocks and nine hens each. Males and females were housed in individual cages. All birds were supplied with fresh water and diet ad libitum. The birds were supplied a ration according to the NRC (1994) contained about 16 % crude protein and 2750 K. Cal-ME / Kg diet (Table 1).

Semen collection and evaluation :

Twice weekly semen collection was initiated up to the 25th week old using the massage technique (training period). Afterward, during which no differences were observed between groups for ejaculate volume and sperm cell concentration. Each group then underwent the following schedule of semen collection per week for six consecutive weeks: Group 1, once (Saturday); Group 2, twice (Saturday and Tuesday); Group 3, three times (Saturday, Monday and Wednesday); Group 4, five times (Saturday to Wednesday) and Group 5, once daily. All ejaculates were collected individually during the experimental period and examined for their quantitative characteristics (volume, concentration, sperm number per ejaculate and daily spermatozoa output) and sperm viability. Ejaculate volume was measured by a tuberculin syring graduated to the nearest 0.01 ml. Sperm concentration was measured by the conventional method using the improved Neubaur Haemocytometer. Total numbers of sperm per ejaculate was estimated by multiplying ejaculate volume x sperm concentration. Comparisons between groups per time unit were performed on a per day basis (daily sperm output). Briefly, DSO was obtained from the formula: total number of sperm collected during 1 week divided by 7 (Brillard and de Reviers, 1985). Alive spermatozoa was checked using semen smears stained with nigrosin-eosin (Lake and Stewart, 1978).

Fertility and hatchability traits :

Semen samples were collected on Saturdays from each group, pooled and diluted (1:3) with 2.9 % sodium citrate extender for artificial insemination. Eighteen Fayoumi hens were assigned per treatment group. Each hen was inseminated once per week, for 8 consecutive weeks breeding period. Every insemination dose contained 100 million sperm. Eggs were collected daily from each group, identified by hen number,

date and then stored at 15 °C for up to 7 days. Eggs were set weekly and fertility was determined by candling at the seventh day of incubation. All removed eggs being broken out to identify any early dead germs that would otherwise have been classified as infertile. Hatchability was determined at the conclusion of hatch.

Statistical analysis:

Data were expressed as the mean \pm SE for all parameters. Data were analyzed by analysis of variance (ANOVA). The differences between group means were tested at least significant difference (LSD). All tests were done using PC-STAT computer program. Results were considered significant at $P < 0.05$.

RESULTS and DISCUSSION

Data in Table (2), exhibit the comparison of the mean values of ejaculate volume, sperm concentration, total sperm / ejaculate, DSO and possible number of inseminations per cock / week between treatment groups. When compared at ejaculate levels (Figure 1), cocks subjected to collection most frequently (i. e., at least three times / wk) had smaller ($p < 0.05$) semen volumes (0.34 to 0.55 ml) than cocks that had semen collected less often (0.63 to 0.64 ml). No significant differences were observed between groups for sperm concentrations, with values ranging between 3.17 and 3.65 billion sperm / ml. Similar results were observed with previous studies (McCartney *et al.*, 1958; Cecil, 1982 and Noirault and Brillard, 1999), increasing the frequency of semen collection had negative effects on ejaculate volume without significantly affecting sperm concentrations, at least when semen collections were performed on a daily basis. Moreover, results in group 2 (twice / week) are in complete agreement with the findings of Dowidar (1999) and Afifi and Dowidar (2000) regarding the mean values of ejaculate volume and sperm concentrations observed in Fayoumi cocks subjected to the same regime of semen collection. On the other hand, McDaniel and Sexton (1977) reported significant differences in both volume and sperm concentration per ejaculate when cocks were subjected to various frequencies of semen collection. In the present study, an explanation for the absence of significant differences in sperm concentrations between ejaculates from Fayoumi cocks subjected to collection at varying frequencies may be attributed to the relatively small number ($n=8$) of males per group due to the high inter-individual variability of semen parameters between cocks within treatment groups. The lowest mean number of sperm per ejaculate was observed in cocks that had semen collected once every day (1.08×10^9 sperm) and the highest in cocks that had semen collected once and twice per week (2.34×10^9 and 2.38×10^9 sperm, respectively). Same results in group 2 were also reported by Dowidar (1999) and Afifi and Dowidar (2000).

When expressed in term of DSO, the mean numbers of sperm per cock per day in each group gradually increased with the frequency of semen collection, ranging between 0.33- to 1.08×10^9 sperm in cocks that had semen collected once / week (group1) to once per day (group 5), respectively. DSO varied in a ratio of 1:3.3 between cocks subjected to one to seven times collection per week. Therefore, the DSO is an important factor when semen is frequently collected and the number of ejaculated sperm per day approaches the DSO. However, because the ductus deferens store spermatozoa, only a portion of these are ejaculated (Cecil and Bakst, 1984 and Cecil *et al.*, 1988). From the comparative standpoint, these results of DSO values in cocks handled 5 to 7 times weekly are similar to those previously observed in turkeys (Noirault and Brillard, 1999), chickens (de Reviere and Williams, 1981) and muscovy ducks (Tan, 1980) subjected to comparable semen collection frequencies.

When considering the possible economic benefits of using males on a high frequency-semen collection schedule, the data in Table (2) were examined from a different standpoints. One of the major goals in any breeding program is selecting a genetically superior sire to inseminate as many females as possible (McDaniel and Sexton, 1977). This goal can be accomplished in several ways, one of which is to obtain the most amount of semen in a given period of time. Thus, the critical consideration is the weekly output of spermatozoa per male. As shown in Table (2), approximately 70 to 75 hens could, hypothetically, be inseminated weekly from each cock undergoing 5 to 7 semen collections per week compared to 23 hens / week if cocks are subjected to one collection per week. Although daily semen collection gave the highest DSO, a little difference was found between five and seven times collection per week (1.00×10^9 versus 1.08×10^9 sperm). This small increase in yield of spermatozoa is unlikely to warrant 1.4 fold the amount of work involved in collecting it. It seems that five times collection / week was the most efficient for production of sperm for artificial insemination during the period under this study.

Table (1) : Composition and calculated analysis of the experimental diet:

<i>Ingredients</i>	<i>(%)</i>
Yellow corn	65.00
Wheat bran	6.63
Soya been meal (44 %)	18.70
Limestone	6.80
Bone meal	2.20
Salt	0.30
Premix *	0.30
Methionine	0.07
Calculated analysis : **	
ME (kcal / kg feed)	2743.95
Crude Protein %	15.88
C : P ratio	172.84
Methionine %	0.34
Lysine %	3.08
Methionine and Cystine %	0.61
Calcium %	3.31
Phosphorus (available) %	0.39

* Each package of 6 kg contains : 5 Million I.U Vit. A.; 1250. I.U Vit. D3; mg Vit. K; 3 gm Vit. B2; 150 mg Choline chloride; 4 gm Calcium D- Pantothenate; 8 mg Vit. B12; Nicotinic Acid 15 mg; 40 mg Magnesium; 1 mg Copper; 1mg Iodine; 100 mg Selenium; 20 mg Iron; 40 mg Manganese ; 20 mg Zinc; 1 mg Cobalt .

** Calculated according to NRC (1994).

Table (2) : Quantitative characteristics of ejaculates collected from cocks submitted to various frequencies of semen collection ($\bar{x} \pm SE$)*

Collection frequency Per wk.	Ejaculate Volume (ml)	Sperm Concentration ($\times 10^9$ /ml)	Sperm Number / Ejac. ($\times 10^9$)	DSO ($\times 10^9$) Sperm	Possible ** Inseminations Per cock / wk
Once	0.64 \pm .14 a	3.65 \pm .12 a	2.34 \pm .31 a	0.33 \pm .06 d	23
Twice	0.63 \pm .12 a	3.78 \pm .08 a	2.38 \pm .22 a	0.68 \pm .10 c	47
Three times	0.55 \pm .11 b	3.57 \pm .09 a	1.96 \pm .15 b	0.84 \pm .10 b	58
Five times	0.41 \pm .08 c	3.42 \pm .12 a	1.40 \pm .10 c	1.00 \pm .11 a	70
Seven times	0.34 \pm .05 c	3.17 \pm .17 a	1.08 \pm .06 d	1.08 \pm .13 a	75

* Means having the same letter in each column do not differ significantly ($P < 0.05$).

** Based on an insemination dose containing 10^8 sperm according to Bilgili *et al.*, 1987.

Table (3) : Daily sperm output (DSO) ; sperm viability percentages and DSO of viable sperm in cocks submitted to various frequencies of semen collection ($\bar{x} \pm SE$)*

Collection frequency per wk.	DSO ($\times 10^9$) Sperm	Viability (%)	Viable DSO ($\times 10^9$ sperm)
Once	0.33 \pm .06 d	87.38 \pm .81 c	0.29 \pm 0.03 d
Twice	0.68 \pm .10 c	87.81 \pm .59 c	0.60 \pm 0.05 c
Three times	0.84 \pm .10 b	88.65 \pm .45 b	0.74 \pm 0.05 b
Five times	1.00 \pm .11 a	90.19 \pm .52 a	0.90 \pm 0.07 a
Seven times	1.08 \pm .13 a	90.23 \pm .54 a	0.97 \pm 0.07 a

* Means having the same letter in each column do not differ significantly ($P < 0.05$).

Table (4) : Frequency of semen collection and egg fertility and hatchability*

Collection frequency per wk.	Fertility (%)	Hatchability (%)	
		Fert. eggs	Total eggs
Once	91	82	75
Twice	94	78	73
Three times	92	85	78
Five times	93	82	76
Seven times	95	81	77
Average	93	82	76

* Fertility and hatchability were obtained from 300 eggs set per group.

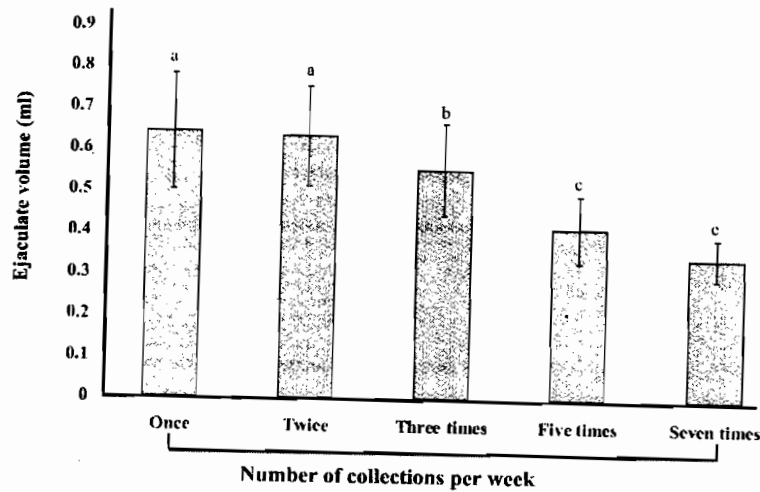


Fig. (1): Mean values of ejaculate volume in Fayoumi cocks collected at various frequencies. Means with no common superscript differ significantly ($P < 0.05$).

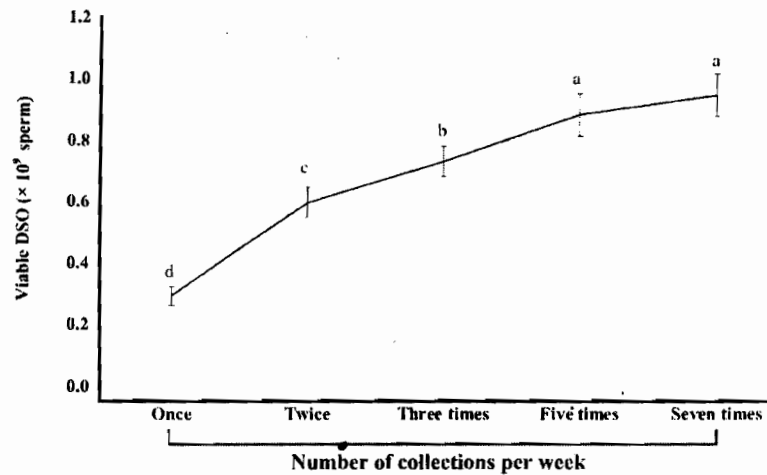


Fig. (2): Mean values of viable DSO in Fayoumi cocks collected at various frequencies. Means with no common superscript differ significantly ($P < 0.05$).

Table 3, shows percentages of viable sperm according to the frequency of semen collection. These results showed significant ($p < 0.05$) increase in the mean percentage of viable sperm in cocks handled more frequently, with values ranging between 87.38% in group (1) and 90.23% in group (5). The results in group (2) parallel the findings of others (Dowidar, 1994 ; El-Ganzory *et al.*, 2001 and Mohamed, 2003) regarding the mean percentage of alive sperm observed in Fayoumi cocks subjected to the same regime of semen collection. Overall, the above results allowed the calculation of a mean viable daily sperm output in each group, which indicated that the mean

number of viable sperm was 3.35 folds in group (5) as that in group (1) (Figure 2). Similar results were reported by Noirault and Brillard (1999) regarding the mean percentage of sperm viability observed in turkey males subjected to the same plan of semen collection. In the present study it has been also observed, in cocks handled daily for 5 consecutive days, then given a two days sexual rest weekly, ejaculates obtained during the days immediately following the sexual rest period contained higher percentages of dead sperm than ejaculates collected during the last days of a given week. The duration of spermatogenesis is constant (7 days) in the fowl (de Reviere, 1968) leading to the conclusion that sperm viability in fowls is at least partly influenced by the duration of *in vivo* sperm storage in the vas deferens. Hypothetically, aging sperm cell may progressively lose membrane integrity due to peroxidation occurring in the male genital tract. Such damage has already been reported in chicken sperm kept *in vitro* for limited periods of times (Surai *et al.*, 1997).

The percentages of fertility and hatchability according to the frequency of semen collection are presented in Table (4). Little differences were observed in the fertility and hatchability of semen of birds subjected to various frequencies of semen collection, with values ranging from 91 to 95 % and 78 to 85%, respectively. These results are in general agreement with that reported for chickens (McDaniel and Sexton, 1977) and turkeys (McCartney *et al.*, 1958). Although some investigators have reported that sperm undergoes certain chemical and physiological changes as a result of repeated ejaculation, no such changes have been correlated with modifications in fertility (Salisbury and Van Demark, 1961; El-Jack and Lake, 1966; McDaniel and Sexton, 1977).

From the results of this study, it can be concluded that varying the frequency of semen collection in Fayoumi cocks not only increases the quantities of sperm available for insemination but also modifies the initial rate of sperm viability in ejaculate. A period of two days sexual rest per week (group 4) aided in the maintenance of high semen yields, this may be necessary to ensure acceptable performance over an entire breeding cycle. Although the various frequencies had no effects on fertility and hatchability in chickens, there still benefits as to collect semen more frequently to inseminate as much females as possible.

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تأثير تكرار جمع السائل المنوي على صفاته الكمية والنوعية والخصوبة والفقس فى الدجاج

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أجريت هذه الدراسة بمزرعة كلية الزراعة _ جامعة الأزهر بأسبوط لمعرفة تأثير تكرار جمع السائل المنوي على صفاته الكمية والنوعية وكذلك المقدرة الإخصابية والفقس فى الدجاج الفيومي حيث إستخدام ٤٠ ديكاً و ٩٠ دجاجة عمر ٢٣ أسبوعاً . قسمت الطيور عشوائياً إلى خمس مجموعات متساوية وتتكون كل مجموعة من مكررتين بكل مكررة أربعة ديوك وتسع دجاجات . وضعت الذكور والإناث فى أقفاص فردية . دربت الديوك على جمع السائل المنوي منها بالتدليك البطني مرتين أسبوعياً ولمدة أسبوعين (فترة تدريب) ، ثم بعد ذلك جمع من المجموعة الأولى مرة واحدة أسبوعياً والمجموعة الثانية جمع منها مرتين أسبوعياً والثالثة ثلاث مرات أسبوعياً والرابعة خمس مرات أسبوعياً والمجموعة الخامسة جمع منها سبع مرات أسبوعياً أى مرة يومياً . وذلك لمدة ستة أسابيع . لتقييم صفات السائل المنوي لكل مجموعة . ثم لفتحت كل مجموعة من الإناث مرة كل أسبوع صناعياً بسائل منوي مجموعة من الذكور بعد تخفيفه بنسبة ١ : ٣ بمخفف سترات الصوديوم وإحتوت جرعة التلقيح لكل دجاجة على مائة مليون حيوان منوي . وذلك لتقدير المقدرة الإخصابية والفقس .

- أوضحت النتائج ما يلى - :

- ١- لوحظ إختلافاً معنوياً ($P < 0.05$) فى حجم القذفة ، فالديوك التى جمع منها مرة أو مرتين أسبوعياً أعطت حجم قذفة أكبر من الديوك التى جمع منها أكثر من مرتين أسبوعياً .
- ٢- لا يوجد فرق معنوى فى تركيز الحيوانات المنوية فى عينات السائل المنوي لديوك المجموعات المدروسة .
- ٣- وجد إختلافاً معنوياً ($P < 0.05$) فى عدد الحيوانات المنوية للقذفة بين جميع المجموعات .
- ٤- أدت زيادة تكرار جمع السائل المنوي من مرة إلى خمس مرات أسبوعياً إلى زيادة معنوية ($P < 0.05$) فى إنتاج الحيوانات المنوية اليومية (DSO) من ٠.٣٣×١٠^٩ إلى ١.٠×١٠^٩ على التوالي بينما لا يوجد فرق معنوى فى قيمة DSO عند الجمع خمس أو سبع مرات أسبوعياً.
- ٥- أوضحت النتائج أنه بزيادة تكرار الجمع تزيد معه كفاءة الديك التناسلية ، فقد وجد انه بزيادة مرات الجمع من مرة الى سبع مرات اسبوعياً تزيد معه عدد الدجاجات التى يمكن تلقيحها صناعياً لكل ديك من ٢٣ إلى ٧٥ دجاجة على التوالي .

- ٦ - لوحظ بزيادة تكرار الجمع من مرة إلى خمس مرات أسبوعياً تزيد معه معنوياً ($P < 0.05$) نسبة حيوية الحيوانات المنوية فى القذفة .
- ٧ - وجد أن متوسط النسبة المثوية للمقدرة الإخصابية كانت ٩٣ % ولا يوجد فرق معنوي بين متوسط % للخصوية وتكرار الجمع .
- ٨ - لوحظ أن متوسط النسبة المثوية للفقس كانت ٨٢ % ، ٧٦ % (منسوبة للبيض المخصب وللبيض الكلى على التوالي) . ولا يوجد فرق معنوي بين % للفقس وعدد مرات الجمع المختلفة .

نستخلص من هذه الدراسة أن زيادة تكرار جمع السائل المنوي من الديوك إلى خمس مرات أسبوعياً يزيد من كفاءتها التناسلية ولا يؤثر عكسياً على المقدرة الإخصابية والفقس فى الدجاج . وينصح باستخدام طريقة الجمع بمعدل خمس مرات أسبوعياً لتعظيم الفائدة خاصة فى حالة القطعان الكبيرة للحاجة الزائدة للتلقيح الصناعي وهذا لن يخفض من نسبتي الخصوية والفقس فى الدجاج .