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EFFECT OF FREQUENCY OF SEMEN COLLECTION, DILUTION RATE AND INSEMINATION DOSE ON SEMEN CHARACTERISTICS AND FERTILITY OF DOMYATI DUCKS

By

A.I.A[1]. Ghonim; A. L. Awad[1]; M. A.El-sawy[1]; M. H. Fatouh[1]; and Zenat, A. Ibrahiem[2]*

1- Anim. Prod. Res. Instit, Agric.Res. Center, Minist. Of Agric. Dokki, Giza.

2-Poult. Prod. Dep. ,Fac. of Agri.,Zagazig Unvi.

E-mail: aymen_ghonim @yahoo.com

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Abstract: *This study was carried out to determine the optimum frequency of semen collections, dilution rate , and artificial insemination dose of Domyati duck semen in order to set up the commercial semen quality which could be applied to the conservation of genetic resources, breeding, and commercial production in Domyati ducks. In experiment 1, the three levels of the frequency of semen collections (once, twice and three times weekly) and dilution rates (raw, 1:1 and 1:2) were studied to determine efficacy of these variables on semen quality, fertility percentage and relative weights of thyroid gland, testis and liver as well as some blood plasma hormones were determined at the end of semen collection period. Experiment 2, was conducted to determine the effects of using different artificial insemination dose (10, 20, and 50 million spermatozoa) on fertility percentage as well as economic efficiency.*

Results showed that the ejaculate volume and sperm concentration were significantly decreased in the thrice semen collections weekly as compared to the once and twice collection. Coiled , clumping and dead sperm percentages were significantly decreased in the twice semen collections weekly and dilution rate 1 : 1 as compared with other treatments, whereas, sperm advanced motility values were significantly increased. Fertility percentage was significantly improved due to interaction between frequency of semen collection twice weekly and dilution rate 1:1, also, it was significantly improved for the groups inseminated by dose containing 20 and 50 million spermatozoa as compared to that contained 10 million sperm. In addition it was significantly improved for the group inseminated

by dose containing 20 million spermatozoa at 6 and 10 days from last insemination as compared to the groups inseminated by dose containing 10 and 50 million spermatozoa.

Thyroid hormones (T_4 and T_3) ,testosterone and insulin –like growth factor hormone were insignificantly increased due to increasing the frequency of semen collections per week . These results indicated that the frequency of semen collections twice weekly with dilution rate 1:1 and an artificial insemination dose being 20 million spermatozoa per duck two times a week , could be used in laying duck insemination to maximize the fertility percentage , in addition to the higher economical efficiency of Domyati ducks.

INTRODUCTION

Artificial insemination in ducks can permit a reduction in the number of drakes needed for breeding. and increase the output of farms. Other avian industries have benefited from artificial insemination by developing breeding lines of desirable traits or using semen of superior males to increase production. The success of this approach will not only depend on our ability to develop systems for semen collection, but also upon the rate of sperm production and the availability of spermatozoa for artificial insemination. To obtain the maximum number of spermatozoa and maintain high rates of sperm production. the semen needs to be collected at a frequency that maintains output over time. In birds, the optimal frequency differs among species and even among breeds of the same species

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs ***(Peters et al., 2004).*** Three sperm parameters that are most often evaluated when determining a male's fertilizing potential included the following: sperm concentration. viability. and motility ***(Bakst and Cecil, 1997).*** In all poultry species, quality parameters change with age of males leading to a progressive decline in fertility ***(Bakst and Cecil, 1992; Kelso et al., 1996)***

The length of fertile period in birds has been defined by ***Lake (1975)*** as the interval between artificial insemination and the last fertile egg laid. The length of this interval depends on the sperm storage in the tubules at the utero-vaginal junction where the spermatozoa are released for movement toward the infundibulum for ova fertilization ***(Brillard, 1993; Brillard et al., 1998)***. In hens, ***Pingel (1990)*** found that the duration of the fertile period

responded to genetic selection, and estimated the genetic parameters of the duration of fertility traits ***Beaumont (1992)***. Domyati ducks is a native Egyptian strain. Recently, it has been a very attracting attention because of its a small-sized and low fat meat. However, the reproductive efficiency of Domyati ducks along with AI techniques are not well investigated.

Therefore, the objective of this study was to determine the optimum frequency of semen collections, dilution rate, and artificial insemination dose and their effects on semen quality, fertility, histological and relative weights of thyroid gland and testis , and blood hormones levels as well as economic efficiency of Domyati ducks.

MATERIALS AND METHODS

This study was carried out at El – Serw Water Fowl Research department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from October 2008 to March 2009. One hundred and eighty nine (162 female and 27 drake) Domyati ducks of 34 weeks of age were used . Birds were reared under similar hygienic and managerial conditions. Drakes were housed in individual cages (50×50×40cm) ,whereas ducks were housed in an open house building as 2.3 ducks /m^2 . Birds received additional artificial light to provide 16 h light and 8 h dark daily . Throughout the experimental period , feed and fresh water were available all the time, except that the drakes were restricted from feed 15 h prior semen collection. The composition and calculated analysis of the breeder diets are shown in Table (1).

Experiment 1:

This trial was carried out to investigate the effect of frequency of semen collection and dilution rate on semen characteristics and fertility percentages after 10 days of incubation . A total number of 27 drakes were randomly chosen and divided into three equal groups , 9 drakes each .All drakes were trained , for three weeks , by abdominal massage technique (***Kammer et al.,1972***) for responding to artificial collection of semen . The frequency of semen collection was once per week (group 1) , twice /week (group 2) and three times / week (group 3). The cloacal region of each drake was washed with warm water and cleaned-dried with a twol . Semen was collected at the base of the copulatary organ in clean and dry graduated tubes. After collection , semen of each group was pooled and then divided into three equal quantities, each quantity was randomly assigned to one of the three dilution rates. The first one was used as fresh non diluted sample ,

while the 2nd and 3rd were diluted with saline(0.9NaCl) solution 1:1 or 1:2 (semen :diluent),respectively .

Semen evaluation:

Semen ejaculates were subjected to evaluate ejaculate volume, spermatozoa concentration, motility, abnormal sperms including coiled-tail and clumped sperms and dead percentages. Semen volume was measured in milliliters using graduated collection plastic tubes. Mass motility was estimated by placing a small drop of diluted or undiluted semen on a slide and immediately examined under the light objective microscope (x10). The scoring system was from 1 to 5 where the bottom, the middle and the top of the scale represent poor, good and excellent motility, respectively (**Etches, 1996**). Advanced motility, progressive movement of spermatozoa, was also determined by microscopic examination(x40) of semen samples diluted with saline (0.9%NaCl) solution. Each sample was ranked for the percentage of moved spermatozoa in straight lines across the filed of vision with a normal vigorous swimming motion .The visual scoring of motion ranged from 0.0 (no motility) to 100 (vigorous motility). Sperm concentration was estimated by using haemocytometer with 1: 200 dilution rate and calculated with the following formula:

Sperm concentration (mm^3) = N x 50 x 200 where, N = number of sperms per 5 large squares of haemocytometer. Percentage of dead spermatozoa was estimated in 200 sperms of different microscope fields by using the nigrosin / eosin staining procedure (***Hackett and Macpherson, 1965***). Sperms that partially or completely stained were recorded as dead spermatozoa. Total abnormalities percentage (primary and secondary abnormalities) was estimated by observing 200 spermatozoa in different microscope fields as described for dead sperms. Percentage of coiled-tail sperms also was estimated and expressed as a percent from abnormalities. The same method also was used to estimate coiled sperm, as reported by ***El-Wardany et al. (1995)*** and ***Tag El-Din et al.(2006)***. Clumping of sperms is a phenomenon of spermatozoa aggregation together, which cause adhering of all sperms lake a pronounced advanced motility.

To examine the fertility percentage, Domyati duck females were randomly distributed into 9 experimental groups (each group was randomly assigned to one of the treatments, then divided into equal three replicates) and starved 15 h before artificial insemination . Ducks were inseminated three times during first week ,then every three days at 10.0 AM by using 0.05 ml raw or diluted (1 :1 and 1 : 2) semen for each semen frequency time . Eggs were collected from each artificial insemination treatment and

incubated. Fertility percentage was determined at the tenth day of incubation by light candling test.

Experiment 2:

This trial was based on the results of the experiment 1 (frequency of semen collection twice weekly) and carried out to investigate the effects of spermatozoa number/dose which used to inseminate one female on fertility percentage. Treatments were artificial insemination by 1 ml fresh diluted semen per duck contained 10, 20 and 50 million spermatozoa twice / week. The equation of ***Etches (1996)*** was used to calculate the number of insemination (N) as follows:

N = volume (ml) X concentration (cells ml^{-1}) / number of cells per insemination. The diluent's used was prepared by mixing 95 ml of saline (0.9Na Cl) solution and 5 ml raw chicken egg yolk and antibiotics were supplemented (50 mg streptomycin + 50000 IU of penicillin) as recommended by ***Sexton et al.1980*** . Also, fertility was estimated at 6 and 10 days after last insemination.

At the end of semen collection period, three drakes from each treatment group were randomly taken for slaughtering. Drakes were fasted for 12 hours before slaughtering and individually weighed pre and after slaughtering until complete bleeding. Presently after scalding, feather picking and evisceration were performed. Testes, thyroid gland and liver were dissected and weighed. During slaughtering, blood samples were collected in heparinized test tubes and centrifuged at 3500 rpm for 15 minutes to obtain blood plasma .Then plasma T_3 and T_4 hormones were determined using radioimmunoassay RIA technique according to ***Akiba, et al.(1982)***. Testosterone hormone was determined as reported by ***Etches and Cunningham (1977)***. IGFS hormone was determined according to ***Goddard et al.(1993)***. For histological examination of testis and thyroid glands, a small piece was taken, preserved in 10 % formaline -saline solution, then dehydrated in alcohol, cleared in zylol and embedded in paraffin wax then sectioned and stained with haematoxyline –eosin stain.

Statistical analysis:

Data obtained were statistically analyzed using the General linear model of ***SAS (1996)***. In this study, two models were used:

Model 1: A 3x 3 factorial designs in experiment 1, considering the

frequency of semen collection and dilution rate as the main effects, as follows:

$Yijk = \mu + Ti + Rj + (TR)ij + eijk$ where : Yijk = An observation ; μ = Overall mean ; T = Effect of frequency of semen collection ; i= (1,2 and 3) ; R = Effect of dilution rate ; j = (1, 2 and 3) ; TR=Effect of interaction between frequency of semen collection and dilution rate ; and eijk = Random error

Model 2: one–way in experiment 2 as follows :

$Yij = \mu + Ti + eiJ$ where, Yij = An observation ; μ = Overall mean ; Ti = Effect of treatment (1, 2, 3) ; and eiJ = Random error .

Significant differences among treatments were estimated by Duncan's multiple range test (***Duncan,1955***).

RESULTS AND DISCUSSION

Semen quality traits:-

Results of Table (2) showed that the frequency of semen collection in Domyati drakes has significantly ($P < 0.01$) affected on ejaculate volume and sperm concentration during experimental period. The highest ejaculate semen volume was obtained by frequency of semen collections at once and twice a week collections. Weekly output values of semen collected volume were increased by about 67.56 and 62.16 % for the frequency of semen collections twice and thrice a week groups as compared to once a week collection group, respectively.

Spermatozoa concentration in the frequency of semen collection three times per week has significantly decreased by 33.33 and 25.0 % as compared to the once and twice a week collection groups, respectively. The highest number of spermatozoa output weekly per drake was improved by the frequency of semen collection increased from once to twice and three times. The values calculated were 2.48×10^9 and 1.8×10^9 sperm for twice and three times as compared by 1.67×10^9 sperm for the collection one time weekly. It is of interest to notice that the ejaculate volume was significantly decreased as the frequency of semen collection increased. A similar trend was observed for the spermatozoa concentration which support the previous results concerning the optimal frequency of semen collection to be three times per week (***McDaniel and Sexton, 1977 and Cooper, 1977***). Our results prefer twice collection per week for good semen and sperm output in Domyati drakes.

The frequency of semen collection and dilution rate has significantly ($P < 0.01$) affected the coiled % , coiled as a percent of total abnormalities and clumping sperm percentages (Table 3). The lowest coiled sperms and clumping percentages of spermatozoa were recorded for the twice weekly collection group of drakes.

The dilution rate with 1 : 1 of drakes semen resulted in a significant decrease in total abnormalities , coiled , coiled / total abnormalities and clumping sperm percentages (Table 3) by about 28.13 ,47.57 ,28.60 and 30.79 % , respectively as compared to raw semen within the three frequency collections ,respectively. The interaction between frequency of semen collection and dilution rate of Domyati drake's semen was significantly affected except for the total abnormalities. The lowest values for total abnormal sperms, coiled and clumping sperm percentages were occurred with semen collection twice a week by dilution rate 1: 1.

Mass and advanced motility of sperm in Domyati drake's semen were not significantly affected by treatments (Table 4), however, the advanced motility score has significantly ($P < 0.05$) decreased due to increasing the frequency of semen collection. Whereas, dead sperms and egg fertility were significantly ($P < 0.01$) affected by treatments with the exception of egg fertility which did not change due to the frequency of semen collection.

It is clear from the results of experiment 1 that the sperm advanced motility values were significantly ($P < 0.05$) higher at frequency of semen collection of once and twice a week as compared to thrice times per week. Dead sperms percentage has significantly ($P < 0.01$) decreased by about 26.30 and 28.91 % for the frequency of semen collection of once and twice as compared to three times weekly, respectively. Whereas, semen dilution rate resulted in significant ($P < 0.01$) decrease in dead sperm percentage by 31.44 and 13.68 % for dilution rate 1:1 and 1:2 as compared to row semen (undiluted), respectively. The interaction between the frequency of semen collection and dilution rate was significantly ($P < 0.01$) affected dead sperm percentage. The lowest value of dead sperm was occurred by frequency of semen collection twice weekly with dilution rate 1:1 .

Fertility of eggs produced after artificial insemination of ducks with different semen treatments was significantly ($P < 0.01$) improved by 5.29 and 7.17 % at dilution rate of 1:1 as compared with undiluted and 1:2 dilution rate , respectively . Good value of egg fertility was occurred by interaction between the frequency of semen collection twice weekly with 1:1 dilution rate. Results declared that semen quality traits were

significantly better in drakes that subjected to twice collection procedure than the other treatments which may reflect the ability of testis to produce and synthesize good quality sperms. This is more obvious in terms of lower numbers of coiled sperms and number of spermatozoa clumps which, together, were the most negative traits affecting fertilizing ability of spermatozoa and hence, the fertility percent. These results are in close agreement with those obtained by ***McCarteny et al., (1958)*** and ***Cecil,(1982)*** who reported a negative effect of frequency of semen collection on abnormal spermatozoa percent. It is also of great importance to notice that the dilution rate of 1:1 gave the best results in terms of lower abnormalities, coiled sperms and clumping of sperms. It is likely that the diluent used in our experiment was efficient to reduce the secondary abnormal sperms and preventing the agglutination of spermatozoa in clumps along with reducing the attachment of coiled-tailed sperms. These results are in close agreement with those reported by ***McDaniel,et al.(1998)*** and ***ParKer and McDaniel(2003)***.

It seems that both advanced motility score and the percent of dead spermatozoa were the most semen quality traits that affected by the frequency of collection, dilution rate and their interaction. This may be due to the genetic background of Domyati drakes and their lower body weight which, perhaps, affect the spermatogenic activity of these males. In this respect ***Garamszegi,et al.,(2005)*** and ***Wingfield and Moore, (1987)*** reported positive correlation between body weight and both semen quality traits in broiler male, which support our results.

Fertility percentage of egg produced after successive artificial insemination during two weeks by different dose of spermatozoa has significantly ($P \leq 0.01$) improved by 47.93 and 22.78% for the group inseminated by dose containing 20 and 50 million spermatozoa as compared with that contained 10 million spermatozoa (Table 5), respectively. Fertility of egg significantly improved for the group inseminated by dose containing 20 million sperms as compared with that of contained 10 and 50 million sperms after 6 and 10 days from last insemination. The present results were in harmony with those reported by ***Etches (1996)*** who observed that 100 million sperm per insemination gave better fertility than 200 or 300 million sperm in both turkey and chicken hens. Generally, fertility percentage decreased by increasing egg collection period after last insemination. It is clear that , the lowest decrease in fertility percentage occurred for the group inseminated with 20 million sperms (9.84 %) as compared to the groups inseminated by dose containing 10 and 50 million sperms (28.21 and 19.08

%) by increasing egg collection period from 6 to 10 days after last insemination .

Concerning the effect of insemination dose on fertility percent, our results indicate that a dose containing 20 million sperms is sufficient to give higher fertility percentage during the whole experimental period (after 6 and 10 days). However, the $10x10^6$ dose was not sufficient to achieve good fertility. Besides, the $50x10^6$ sperms dose was not recommended under our experimental condition. It is suggested that, the 20 million sperms in the same volume (1ml) are more active because sperms were able to move freely and quickly, and to utilize the energy sources of diluent more efficiently than the dose of 50 million sperms. This may allow them to reach and occupy the sperm host glands in the tubo-uterine junction and increase the fertility percentage.

Relative weight of thyroid gland was decreased by 23.07 and 7.92 % for the frequency of semen collections twice and three times weekly (Table 6), whereas, relative weights of testis were increased by 12.90 and 9.68 % for the same groups as compared to the once collections group per week , respectively . Relative weight of liver was significantly decreased by 33.3 % for both the frequency of semen collections twice and three times weekly as compared to the once collections group.

It is observed that the relative weight of thyroid gland was greatly reduced with increasing the frequency of semen collection. At the same time this coincident with the significant decreases in the relative weight of the liver. It appeases that the frequency of semen collection may stimulate thyroid gland to secrete its hormones in excess and this increase could affect their size and weight. If this is the case, it may explain the reduced liver weight as the liver is the main metabolic organ in the body that responds to the nutrients demands for the progressive spermatogenic activity in the groups of twice and three times frequencies. The previous results were reflected on the testis weight which was increased as a result of the stimulation effect of semen collection. It is likely that a pituitary – thyroid – testis axis was established in response to the frequency of massage for semen collection. These results are in close agreement with the basic knowledge of the reproductive physiology as reported by ***Sturki (2000)***, and ***Etches (1996)***.

Thyroid hormone (T_4) increased by 16.75 and 11.82 % for the frequency of semen collections twice and three times weekly (Table 7), whereas, thyroid hormone (T_3) increased by 8.89 % for the same groups as compared to the frequency of semen collection once weekly, respectively.

Testosterone hormone was insignificantly increased by 18.52 and 25.93 % for the frequency of semen collections twice and thrice weekly as compared to the one time collection weekly, respectively. Insulin-like Growth factor hormone was insignificantly increased by 3.72 and 13.83 % for the frequency of semen collections twice and thrice weekly as compared to the once weekly collection, respectively.

Non significant effects of semen collection frequency of Domyati drakes were observed on neither thyroid hormones (T_3 and T_4) nor testosterone hormone. Our results indicate that the turnover of T_4 to T_3 was higher in treatment groups. There is a positive relationship between thyroid hormones and testosterone level was also recorded. Moreover, IGFs behaves with the same trend as enhancing the reproductive performance of drakes. These results agree with the findings of many workers who used different avian species including ducks, and found a positive correlation between the endocrine glands and different semen traits (***EL-Wardany.et al.,1995, Brillard,et al,1998; Mclachan, et al.,1996; and Kirby and Froman,2000***).

Thyroid gland histology:

The histological structure of the thyroid gland as influenced by the frequency of semen collection showed considerable changes (plates 1 to 3). Thyroid follicles of the once semen collection group were normal in their diameter and colloid contents (plate 1). The epithelial linning of these follicles tended to be cuboidal indicating euthyroidism status, however, careful examination showed normal active follicles, resulted from the low – columnar appearance of their epithelial linning. This may indicate that collection of semen once/ week did not require hyperfunction of the endocrine glands. It is of interest to observe the histological changes in thyroid histology of the twice and thrice collections groups (plate 2 and 3) ,the follicles became larger, filled with colloid, and their epithelial linning was columnar- shaped indicative of hyper activity of the gland. However, some follicles in the latter group showed an irregular or elongated follicle containing lesser amounts of colloidal materials indicative of a stressful condition resulting from increasing the frequency of semen collection. These results may suggest a higher metabolic rate to supply the demand organs with their needs, during the spermatocytogenesis. The relationship between thyroid gland and the testis development is well known and thyroid status has a great effect on semen production and semen quality via its effects on the nutrients and nucleic acids needed for semen production and spermatozoa metabolism. Our results agree with those reported by ***El-Wardany et. al.(1995) , Bakir,1981 and Sturkie,(2000).***

Testis histology:

Histological examination of the testis sections revealed interesting observations (plates 4 to 6). The testicular tissue of the group one (once semen collection/ week) showed semineferous tubules (ST) of different size enclosed in a connective tissue capsule containing fine elastic fibers (Plate 4). From the periphery towards the lumen are found progressively more mature spermatogonia, primary spermatocytes, secondary spermatocytes, and finally spermatids and mature spermatozoa in the lumen. Within the epithelium linning of ST many sertoli cells are present. Plate 5 and 6 showed a considerable change in ST shape and diameter, the number of mature spermatozoa attached sertoli cells or packed in the lumen of ST cells. These structures were more relevant in the drakes of group two (twice collections) where the histological structure of ST being more active and contained different stages of spermatogenic process (plate 5). This case was less relevant in group three which may suggest that three times of semen collection / week may disrupt the spermatogenesis as depicted in the histological appearance (plate 6) and hence, more abnormal sperms may be produced. This holds true as our results revealed that this group showed higher numbers of abnormal and dead spermatozoa along with coiled-tail and clumps of sperms (Table 3 and 4). It is likely seems that twice semen collections per week from Domyati drakes is a very good procedure to obtain good quality semen and to keep healthy- unstressed drakes as indicated by their thyroid and testis histological sections and their hormonal profile presented in the study. Previous findings suggest 2 to 3 times of semen collection a week to be a suitable management for turkey and broiler breeder males (*Etches, 1996).* As the authors have been able to review the literature, there have been no earlier reports concerning the effect of semen collection frequency on the physiological and histological status of drakes in general and in Domyati drakes especially. Therefore, further researchers are needed to clarify and support our observations.

CONCLUSION

From our field observations and these physiological markers, it is suggested to collect semen twice weekly and to use a dilution rate of 1 : 1 for enhancing the fertility of the artificially inseminated Domyati ducks. From the economical point of view it is concluded that a dose of 20 x 10^6 spermatozoa / insemination is sufficient to improve fertility percentage. This means that an ejaculate volume of 0.31 ml could be used to inseminate 58 females weekly, ie. 4640 females per production season (40 weeks), which reflect sparing effect of using artificial insemination technique in Domyati ducks.

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

Ingredients	%
Yellow corn	66.00
Soya bean meal (44 %)	21.50
Wheat bran	2.70
Di-calcium phosphate	1.50
Limestone	7.60
Vit & Min. premix *	0.30
Salt (NaCl)	0.30
DL. Methionine (97%)	0.10
Total	100.0
Calculated Analysis **	
Crude protein	15.50
ME (Kcal / kg)	2724
Calcium	3.410
Available phosphorus	0.45

* Each 3kg of Vit .and Min. premix contains 100000000 IUVit A;2000000 IU Vit.D3;10 g Vit.E; 1 g Vit.K ; 1 g Vit B1; 5 g Vit B2 ;10 mg Vit.B12 ; 1.5 g Vit B6; 30 g Niacin ;10 g Pantothenic acid ;1g Folic acid;50 mg Biotin ; 300 g Choline chloride; 50 g Zinc; 4 g Copper; 0.3 g Iodine ; 30 g Iron; 0.1 g Selenium ;60g Manganese ;0.1 g Cobalt; and carrier $CaCo_3$ to 3000 g .

** According to NRC (1994)

Table (2): Effect of frequency of semen collection per week on semen volume and concentration of Domyati duck drake.

Items	Frequency of semen collection(times/ week)			Sig.
	Once	**Twice**	**Thrice**	
Ejaculate volume (ml) / drake	0.37± 0.02 [a]	0.31± 0.02 [a]	0.20±0.02 [b]	0.01
Concentration of sperms ($x10^9$)/ ejaculate	4.5±0.3 [a]	4.0±0.2 [a]	3.0±0.1 [b]	0.01

a,b :means in the same row bearing different superscript are significantly different ($P \leq 0.05$).

Table (3): Effect of frequency of semen collection and dilution rate on some semen characteristics of Domyati drakes.

Treatments		%			
		Total Abnormal.	Coiled	Coiled / TA	Clumping
Frequency of semen collection (time / week)					
Once		10.8±0.5	5.44±0.44 ^{b}	50.00±2.72 ^{ab}	7.44±0.47 ^{ab}
Twice		10.9±0.8	5.22±0.76 ^{b}	46.11±4.77 ^{b}	7.00±0.52 ^{b}
Thrice		12.2±1.0	6.56±0.60 ^{a}	53.45±2.29 ^{a}	8.33±0.50 ^{a}
Significance		NS	**0.01**	**0.05**	**0.01**
Dilution rate					
Raw		12.8±0.7 ^{a}	7.00±0.33 ^{a}	55.10±1.30 ^{a}	8.67±0.44 ^{a}
1 :1		9.2±0.9 ^{b}	3.67±0.41 ^{b}	39.34±3.82 ^{b}	6.00±0.29 ^{b}
1 :2		11.9±0.1 ^{a}	6.56±0.41 ^{a}	55.12±1.34 ^{a}	8.11±0.35 ^{a}
Significance		**0.01**	**0.01**	**0.05**	**0.01**
Interactions					
Freq.	Dil.				
Once	**Raw**	12.3±0.3	6.67±0.33 ^{ab}	54.03±2.39 ^{abc}	8.00±1.15 ^{abc}
	1 :1	9.3±0.3	4.00±0.58 ^{d}	42.97±6.60 ^{c}	6.33±0.33 ^{de}
	1 :2	10.7±0.5	5.67±0.33 ^{bc}	53.00±1.49 ^{abc}	8.00±0.57 ^{abc}
Twice	**Raw**	12.7±1.3	7.00±0.58 ^{ab}	55.70±2.97 ^{ab}	8.67±0.33 ^{ab}
	1 :1	8.3±0.7	2.33±0.33 ^{e}	28.03±3.21 ^{d}	5.00±0.01 ^{e}
	1 :2	11.7±0.9	6.33±0.33 ^{ab}	54.60±2.91 ^{ab}	7.33±0.33 ^{bcd}
Thrice	**Raw**	13.3±2.03	7.33±0.88 ^{a}	55.57±2.23 ^{ab}	9.33±0.67 ^{a}
	1 :1	10.0±0.6	4.67±0.33 ^{cd}	47.03±4.57 ^{bc}	6.67±0.32 ^{cde}
	1 :2	13.0±1.7	7.67±0.88 ^{a}	57.77±2.23 a	9.00±0.58 a
Significance		NS	**0.01**	**0.01**	**0.01**

a,b,c,d,e :means in the same column bearing different superscript are significantly different ($P \leq 0.05$).

Table (4): Effect of frequency of collection and dilution rate on some semen characteristics of Domyati drakes and fertility of eggs produced.

Treatments		%			
		Mass motility	**Advanced motility**	**Dead spermatozoa**	**Fertility**
Frequency of semen collection (time / week)					
Once		4.90±0.03	95.67±0.33[a]	6.22±0.62[b]	87.66±1.00
Twice		4.89±0.03	95.00±0.52[a]	6.00±0.37[b]	88.37±1.58
Thrice		4.67±0.17	91.67±1.44[b]	8.44±0.50[a]	86.88±1.04
Significance		**NS**	**0.05**	**0.01**	**NS**
Semen dilution					
Row		4.82±0.10	94.22±1.22	8.11±0.61[a]	86.63±0.59[b]
1 :1		4.81±0.11	94.33±0.92	5.56±0.44[c]	91.22±0.81[a]
1 :2		4.83±0.11	93.78±1.14	7.00±0.50[b]	85.11±0.97[b]
Significance		**NS**	**NS**	**0.01**	**0.01**
Interactions					
Freq.	Dil.				
Once	**Raw**	4.90±0.06	96.00±0.57	8.00±1.15[abc]	86.90±0.77[bcd]
	1 :1	4.92±0.04	96.00±0.58	5.00±0.58[ef]	90.27±0.73[ab]
	1 :2	4.88±0.07	95.00±0.57	5.67±0.67[def]	85.80±1.02[cd]
Twice	**Raw**	4.90±0.06	95.00±1.15	6.67±0.33[cde]	86.27±1.00[bcd]
	1 :1	4.87±0.08	95.33±1.20	4.67±0.32[f]	93.60±0.21[a]
	1 :2	4.92±0.04	94.67±0.88	6.67±0.33[cde]	85.23±1.42[d]
Thrice	**Raw**	4.67±0.33	91.66±3.33	9.67±0.88[a]	86.53±0.78[bcd]
	1 :1	4.66±0.31	91.67±1.67	7.00±0.58[bcd]	89.80±0.77[abc]
	1 :2	4.67±0.32	91.66±3.30	8.67±0.33[ab]	84.30±0.73[d]
Significance		NS	NS	**0.01**	**0.05**

a,b,c,d ,e,f :means in the same column bearing different superscript are significantly different ($P \leq 0.05$).

Table (5): Effect of the spermatozoa number / dose on fertility of incubated eggs.

Treatments (million spermatozoa/ dose)	Fertility %		
	During successive AI	After 6 day from last AI	After 10 day from last AI
10	63.63 ±1.76[c]	63.33±1.93[b]	45.46 ±1.07[b]
20	94.13±1.70[a]	68.77±1.82[a]	62.00±1.15[a]
50	78.13±1.82[b]	58.33±2.39[b]	47.20±1.61[b]
significance	0.01	0.01	0.01

a,b,c, :means in the same column bearing different superscript are significantly different ($P \leq 0.05$).

Table (6): Effect of frequency of semen collection per week on weight and relative weight of thyroid, liver and testis of Domyati drakes .

Items		Frequency of semen collection(times/ week)			Sig.
		Once	Twice	Thrice	
LBW g		1813.5±78.4	1953.3±32.8	1853.3±32.8	NS
Thyroid	G.	0.47±0.07	0.37±0.12	0.41±0.10	NS
	%	$3x10^{-4}$±$4x10^{-5}$	$2x10^{-4}$± $6x10^{-5}$	$2x10^{-4}$± $5x10^{-5}$	NS
Liver	G.	24.3±1.1 ^{a}	19.1±0.9 ^{b}	22.5±0.8 ^{a}	0.01
	%	$13x10^{-3}$±$3x10^{-4}$ ^{a}	$10x10^{-3}$ ±$2x10^{-3}$ ^{b}	$12x10^{-3}$± $5x10^{-4}$ ^{a}	0.01
Testis	G.	56.4±3.9	68.5±13.1	61.7±10.0	NS
	%	$31x10^{-3}$ ± $2x10^{-3}$	$35x10^{-3}$ ± $6x10^{-3}$	$34x10^{-3}$± $1x10^{-3}$	NS

a,b,c,d :means in the same row bearing different superscript are significantly different ($P \leq 0.05$).

Table (7): Effect of frequency of semen collection per week on some blood hormones of Domyati drakes.

Items	Frequency of collection (times/ week)		
	Once	Twice	Thrice
T_4 (ng/dl)	20.3±1.0	23.7±1.3	22.7±0.8
T_3 (ng/dl)	4.5±0.3	4.9±0.2	4.9±0.1
Testosterone (ng/dl)	2.7±0.3	3.2±0.5	3.4±0.1
IGF_s	233.6±25.2	242.3±16.4	265.9±16.8

All parameters are not significantly different ($P \leq 0.05$).

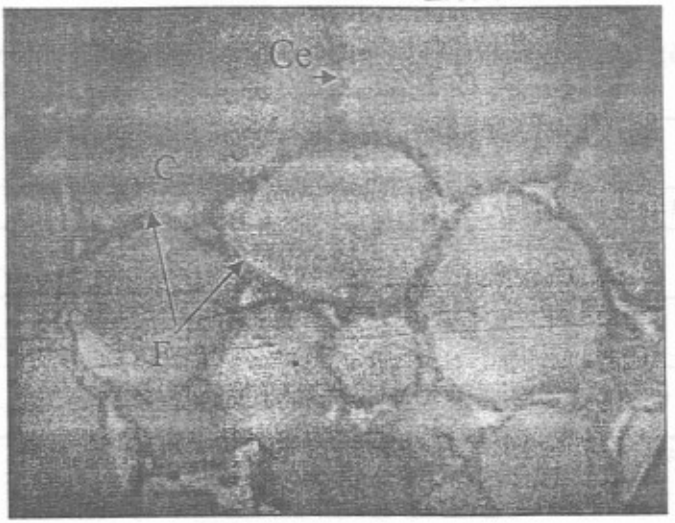
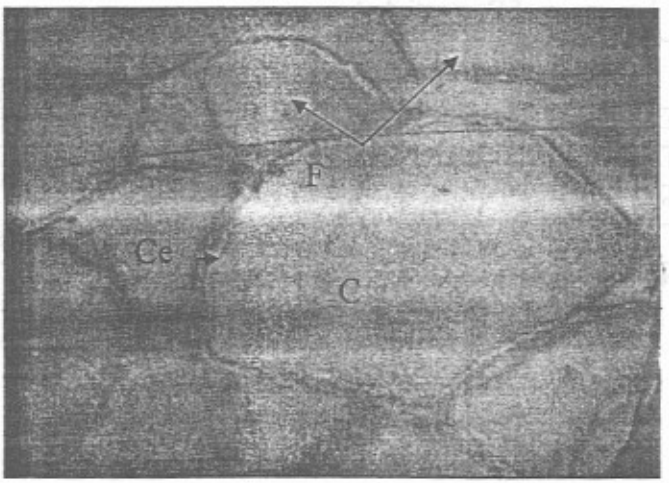


Plate.(1): T.S. in the thyroid gland of the drakes in group one (H&E x200).



Plate(2): T.S.in the thyroid gland of the drakes in group two (H&E x200).

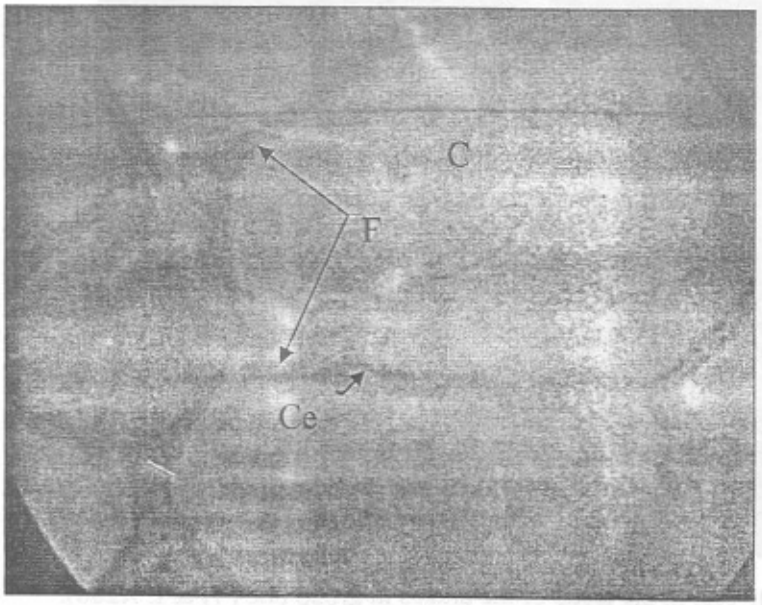


Plate.(3): T.S. in the thyroid gland of the drakes in group three (H&E x200).

Abbreviation key :

C : colloid

Ce : epithelial linning

F : thyroid follicle

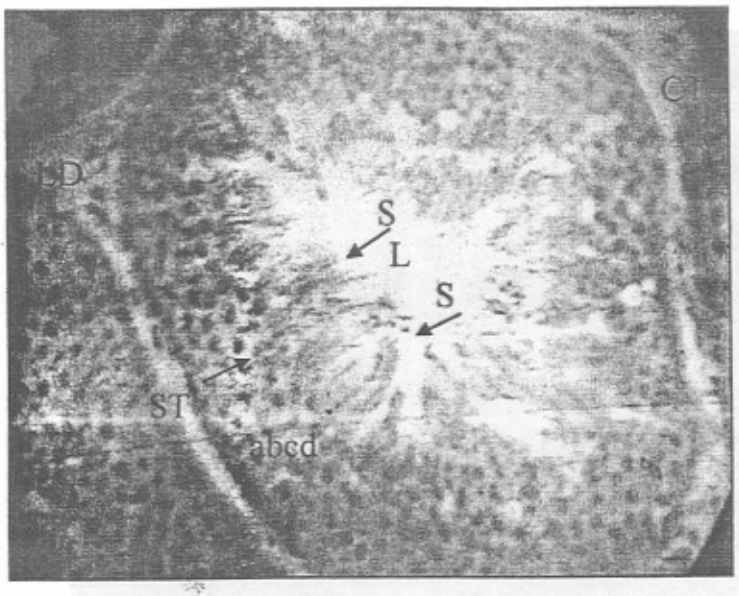


Plate (4): T.S. in the testis of the drakes in group one (H & E X200).

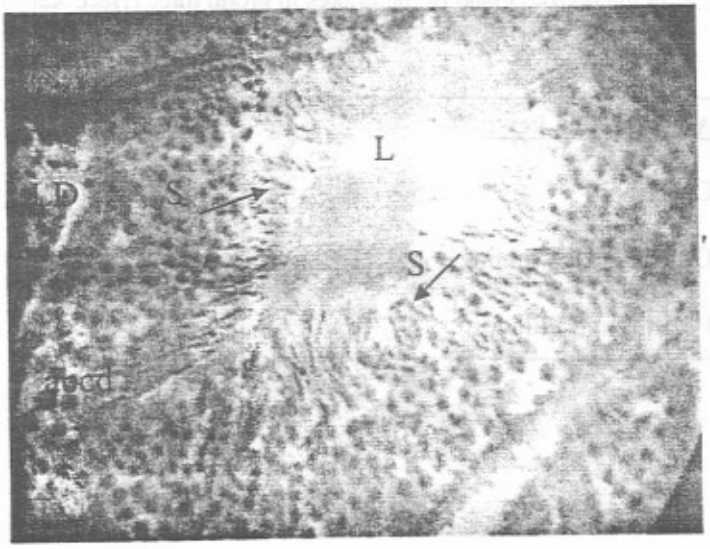


Plate (5):T.S. in the testis of the drakes in group twos (H&EX200).

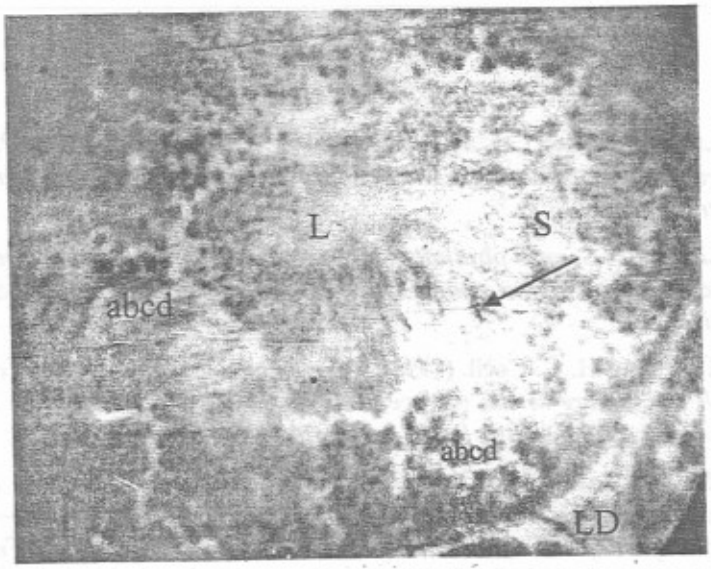


Plate (6): T.S. in the testis of the drakes in group three (H&E X200).)

Abbreviation key :

ST : semineferous tubule
CT : connective tissue capsule
L : lumen
S : spermatozoa
A,b,c,d :spermatogonia 1ry,2ry spermatocytes and spermatids, respect.
LD : leydig cells

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الملخص العربى

تأثيرعدد مرات جمع السائل المنوى ومعدل التخفيف وعدد الاسبرمات بجرعة التلقيح الاصطناعى على صفات السائل المنوى والخصوبة فى البط الدمياطى

أيمن إبراهيم عبده غنيم[1] ، عوض لطفي عوض[1] ، محمد عبدالعزيزالصاوى[1] ، محمد حسن عبدالعزيز فتوح[1] ، زينات عبدالجواد ابراهيم[1]

١- معهد بحوث الإنتاج الحيواني ـ مركز البحوث الزراعية ـ وزارة الزراعة ـ الدقي ـ جيزة ـمصر

٢- قسم انتاج الدواجن ـكلية الزراعة- جامعة الزقازيق- مصر

أجريت هذه الدراسة لتقدير أنسب عدد من مرات جمع السائل المنوى ومعدل التخفيف وعدد الحيوانات المنوية المستخدمة فى كل تلقيحة لسلالة البط الدمياطى للوصول الى أحسن صفات للسائل المنوى ونسبة الخصوبة التى يمكن استخدامها وتطبيقها فى تحسين انتاجية هذه السلالة .

تم استخدام عدد ١٨٩ طائر بط دمياطي عمر ٣٤ أسبوع (١٦٢ بطة و ٢٧ ذكر) فى المعاملات التجريبية ، التجربة الأولى استخدم فيها عدد ٢٧ ذكر بط دمياطى قسمت الى تسع معاملات فى ثلاث مكررات لدراسة تأثير عدد مرات جمع السائل المنوى (مرة واحدة ، مرتين ،ثلاث مرات) أسبوعيا فى ثلاث مستويات للتخفيف (بدون تخفيف ، ١ :١ ، ١:٢) لكل مستوى من الجمع على جودة السائل المنوى ونسبة الخصوبة ، أما التجربة الثانية فقد صممت لدراسة تأثير محتوى جرعة التلقيح الاصطناعى من الحيوانات المنوية (١٠ ، ٢٠ ، ٥٠ مليون لكل بطة مرتين أسبوعيا) على نسبة الخصوبة ، وسجلت الأوزان النسبية للغدة الدرقية والخصيتين والكبد وتم تقديرمحتوى البلازما من بعض الهرمونات فى نهاية فترة جمع السائل المنوى ،بالاضافة لعمل قطاعات هستولوجية على الغدة الدرقية والخصية لدراسة التركيب الخلوى لهما .

وكانت النتائج المتحصل عليها :-

١- زيادة كمية السائل المنوى الناتجة أسبوعيا وكذلك عدد الاسبرمات بزيادة عدد مرات الجمع الأسبوعية، بينما انخفضت نسبة الاسبرمات المشوهة والميتة معنويا بجمع السائل المنوى مرتين أسبوعيا مع استخدام معدل تخيف ١ : ١ .

٢- تحسنت نسبة الخصوبة معنويا بجمع السائل المنوى مرتين أسبوعيا مع استخدام معدل تخفيف بنسبة ١ : ١ . وكذلك تحسنت نسبة الخصوبة معنويا للبيض الناتج من المجموعة التى لقحت اصطناعيا بجرعة تحتوى على ٢٠ مليون اسبرم لكل بطة مرتين اسبوعيا واستمر هذا التحسن معنويا بعد ٦ أيام و ١٠ أيام من آخر تلقيح بالمقارنة بالبيض الناتج من المجموعات التى لقحت بجرعات تحتوى على ١٠ و ٥٠ مليون اسبرم .

٣- انخفض الوزن النسبى لكل من الغدة الدرقية والكبد بينما ارتفع الوزن النسبى للخصيتين بزيادة عدد مرات الجمع كما ازدادت محتويات البلازما من هرمونات الدرقية وهرمون التستوستيرون وكانت الزيادة غير معنوية بينما هرمون IGFS كانت الزيادة فيه معنوية بزيادة عدد مرات جمع السائل المنوى من مرتين الى ثلاث مرات أسبوعيا .

٤- توضح القطاعات الهستولوجية زيادة فى نشاط الغدة الدرقية وزيادة حجم النسيج الطلائى لها بالاضافة الى حجم السائل الغروى داخل الحويصلات وذلك بزيادة عدد مرات جمع السائل المنوى، كما توضح وجود نشاط كبير لعملية تكوين الحيوانات المنوية وبصفة خاصة عند جمع السائل المنوى مرتين أسبوعيا.

وبناء على النتائج السابقة ومن خلال الملاحظات الحقلية نستنتج أن جمع السائل المنوى مرتين أسبوعيا وتخفيفه بنسبة ١:١ يمكن أن يساعد على تحسين صفات جودة السائل المنوى مع استخدام عدد ٢٠ مليون حيوان منوى فى التلقيحة الواحدة مرتين أسبوعيا لكل بطة حيث أن ذلك يؤدى الى تحسن فى نسبة الخصوبة بالاضافة الى الكفاءة الاقتصادية لسلالة البط الدمياطى المحلية .