

# Studies of Semen Qualities of Lahore Pigeons (Columba Livia)

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## Abstract

Ejaculation capabilities and semen characteristics depending on seasonal variations are presented in this paper. The mean temperature range in the study area was 25.6°C (February) -30.5°C (May) and the mean humidity range was correspondingly between 62.5%–80.4

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**Index terms**— lahore pigeon, semen quality, semen ejaculation and season.

## 1 I. Introduction

reproductive performance of male pigeons is directly related to the semen quality as has been recognized in the poultry science that for selecting breeding males or for routinely monitoring their reproductive performance, evaluation of semen is of the intrinsic worth in poultry breeding. The analysis of pigeon's semen characteristic has hardly been possible due to the lack of adequate information about the qualities of the semen. The semen has been collected from birds such as drakes (Setioko and Hetzel, 1984), cocks (Saeid and Al-Soudi, 1975, Lake and Stewart, 1978), ganders (Pawluczuk and Grunder, 1989), turkeys (Burrows and Quinn, 1937; Noirault and Brillard, 1999), pheasants (Mantovaniet al., 1993) and racing pigeons (Cheng et al., 2002) and its qualities have been evaluated in the breeding industry (Sexton, 1977; Setioko and Hetzel, 1984). Manual massage is the most frequently used method of semen collection from fowls and turkey (Burrows and Quinn, 1937) and pigeons (Cheng et al., 2002), but use of artificial vagina in ganders (Pawluczuk and Grunder, 1989) and electro ejaculation in drakes (Setioko and Hetzel, 1984) were also described systematically. The first paper regarding the yield of pigeon semen by manual massage was documented as early as 1941 (Owen); this method is safe, undisruptive, and not stressful to the donor. Cheng et al (2002) have evaluated the annual change in the semen qualities of racing pigeons but there is no report regarding the semen characteristic of Lahore pigeons so far. Since racing pigeons take more amount of feed than the Lahore pigeons, the semen qualities of the latter would possibly vary from the former.

## 2 R

The semen of birds has a significant effect on their fertility and reproductive potential (Sexton, 1983), which is affected by environmental conditions (McDaniel et al., 1995; McDaniel et al., 1996) and nutrition provided to the birds (Athis Kumar and Anatha Rajan, 2016). Similar studies conducted in domestic fowls reveal that semen quality is influenced by seasons (Saeid and Al-Soudi, 1975). Likewise, ambient temperature greater than 31°C notably affects the rooster sperm motility, viability, and fertilizing potential of domestic fowls (McDaniel et al., 1996). Further, change in atmospheric temperature at ejaculation is an important exogenous physiological factor that affects the sperm motility (Ashizawa and Sano, 1990; Wishart and Wilson, 1999). There was a negligible effect of a high ambient air temperature (50-60°C) on the breeding potential of rock pigeons (Arieli et al., 1988) in the nature, but deviation in the ejaculation performance of males in response to ambient temperature has not been made clear. The increase in fertility percentage of eggs is related to higher sexual efficiency and better semen quality of males (Mariety, 2005). The quality and quantity of semen produced by birds are the deciding factors for the fertility of eggs (Sexton, 1983), which usually varies depending on environmental conditions (McDaniel et al., 1995). According to Owen (1941), the semen quantity of domestic pigeons is not at all related to the size of the bird but it appears to be related to the seasons of year. The semen is of superior quality during the spring and summer, so that the fertility of eggs is relatively high during these two seasons (Cheng et al., 2002; Athis Kumar, 2017).

Evaluation of semen qualities of Lahore pigeons has hardly been known so far. The semen quality evaluation in Lahore pigeons (*Columba livia*) and ejaculation performance in terms of submission of male birds and the colour of their cloacal mucosal secretion during collection were investigated and presented in this paper.

## II. Materials and Methods

### a) Experimental Design

Two year old male Lahore pigeons (*Columba liviadomestica*; family: Columbidae; order: Columbiformes) weighing about 350g were chosen as the experimental birds for this study and grown in the Animal House at the Zoology Department of Sivanthi Anthithanar College situated at 77°13'E and 8°29'N in Tamilnadu. 20 pigeons were housed in separate lofts of 40 x 30 x 50cm size. The lofts were constructed with wooden frame, steel plated roof and wire mesh floor and lateral sides. These lofts were kept at a height of 2.5' from the ground level for reducing dampness facilitating the rapid spreading of pathogenic germs. All the lofts were equipped with a wind insulation to the north under native environmental conditions (24°07' N, 120°40'E), where birds received a natural photoperiod of 12L: 12D to 15L: 9D throughout the experiment. Feed mixture (in Table 1) was given at the rate of 45 grams / bird / day and drinking water was provided at the rate of 60 ml/ bird/day. Vitamins required for the birds were provided along with the drinking water at the rate of 5ml of Vimeral® (vitamin mix)/ 1 liter water. This feed composition was maintained throughout the study period for feed uniformity in the experimental pigeons groups. Pigeons were maintained in the lofts for 2 weeks period to enable them to adjust the experimental conditions and to be friendly enough to the operator.

Semen collection was performed for over a 2-weeks period and those pigeons which did not produce semen by the end of the training period were excluded from the experiment. Finally, 10 pigeons with optimal ejaculation performance were used throughout the study. Semen

### b) Semen Collection

Semen collection was performed from each and every bird all the times only by the investigator to avoid variations in handling performance of pigeons. The technique described by Owen (1941) was employed while collecting the semen. The pigeon was caught and held with its chest positioned against the collector's belly. Tail feathers around the cloaca were clipped before semen collection to prevent contamination of the ejaculate and to have clear view of the cloacal opening. Its wings were stretched and then closed to relax the birds while fixing the main feathers in order. Afterward, massage was exerted by applying pressure with thumbs and index fingers, up and down the exterior of the pygostyle. Thumbs were rhythmically pressed on the rump region, while the index fingers massaged the opposite abdominal region in the vicinity of cloaca for a few rounds. Concurrently, the middle finger of the right hand pressed the os pubis, just below the vent. If the positions of the fingers were correct, the cloaca opened wide showing two fleshy folds. Then, the little finger on the left hand was hooked under the right wing while the other fingers were extended along the donor's back. The donor was then turned onto its back and lay on the left palm. The tail of the pigeon was free to move and spread. Thereafter, the thumb and index finger of the left hand were rhythmically pressed against the opposite sides of the caudal bone. The milking action of thumb and index finger could exert on the base of the cloacal projection while the middle finger was pressed deeply between the pubis bones. After 2 seconds of massage, a small drop of white semen flew from the opening of the cloaca. Massage was continued for about 30 seconds and semen was aspirated into a micropipette equipped with a fine tip. The quantity of semen was directly measured with the micropipette. If there was no ejaculate within 30 seconds, the donor was returned to his loft, and a second attempt was made after 15 min.

### c) Submission Test

Responses of the pigeons while collecting the semen were noted and classified into submissive, tolerant, and resistant types. In the submissive response, pigeon was calm and waving the tail to show the cloacal opening; in the tolerant response, it was slightly struggling but allowed semen collection; and in the resistance, pigeon struggled by flapping and attempted to escape. was collected from each male pigeon twice in a week (Monday and Thursday) between 8.00 AM and 10.00 AM.

### d) Testing the Cloacal Mucosa Colour

The colour of the cloacal mucosa was tested at the time of massaging for semen collection. The colour of the everted membrane folds of cloacal opening was recorded, which was either red or pink or pale depending on the redness of the membrane. impermeable to eosin and dead cells which were permeable to eosin were assessed from the total count of 100 spermatozoa/ slide.

### f) Statistical Analysis

Data obtained from this experiment was subjected to one-way ANOVA, using SPSS (1997) computer software. The significant differences among the means of different dietary treatments were analyzed with the Duncan multiple range test (Duncan, 1955).

## 9 III. Results

Seasonal variations in the ejaculations and semen collection rates are shown in the table-1. The mean temperature range in the study area was 25.6°C (February) -30.5°C (May) and the mean humidity range was correspondingly between 62.5-80.4% (February) and 77.6-83.3% (May). The photoperiod was minimum in February (13h/day) and maximum in May (15.6h/day). Out of 927 collection attempts, 509 attempts ended with successful ejaculation and hence the semen collection rate was 54.9±8.8%. The highest collection rate was 65% (52/80) in March followed by 63.7% (51/80) in November, whereas significantly lowest collection rate was 44.7% (34/76) in September. Table-2 clearly depicts the seasonal changes in the semen characteristics of Lahore pigeons. Average volume of semen ejaculated from a bird in one massage collection was 9.3±1.5µl and the range was 6.1 ± 0.2 µl (September) -10.8 ± 0.7 µl (November)/ collection ( $p>0.05$ ). The average semen concentration was 4.01±0.5x10<sup>9</sup> /ml. The highest semen concentration (4.5±0.3x10<sup>9</sup> /ml) was observed in March and November and the least semen concentration was observed in July (3.4±0.2x10<sup>9</sup> /ml). The statistical significance was  $p<0.05$ . The mean value of sperm motility was 76.3±8% per ejaculate, the highest motility (83±3%) was noted in March and November ( $p<0.05$ ) while the lowest motility (70±2%) was in July and October ( $P>0.05$ ). The average sperm viability was 71.8±10.2% and the viability range was 61±3% (September) -78±5% (March and November). The statistical significance was  $p<0.05$ . Sperm viability and sperm motility were positively correlated ( $r=0.94$ ;  $P<0.05$ ). The overall ejaculation performance was best during March and November compared to other months of a year but very poor in September. There were 5 -16 granular crystals of uric acid per ml of semen, which were in large proportions during March ( $p>0.05$ ) and least proportion e) Semen Quality Evaluation Volume of semen was measured directly when it was aspirated from the cloacal vent using a micropipette. The semen was examined under a compound light microscope for contamination with blood, feces, and uric acid. To estimate the sperm motility, semen samples were diluted 20-fold using Beltsville poultry semen extender (Sexton, 1977) and 10µl of the diluted semen was taken and placed over 10 slides which were then covered by a cover glass (18 x 18 mm) and viewed under the microscope. Motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive movement. To estimate the sperm concentration, semen was diluted to 1:200 with a weak eosin solution (Brillard and McDaniel, 1985) and sperm cells were counted using a hemocytometer. Sperm morphology was examined microscopically (500x) in smears stained with nigrosin and eosin (Blom, 1950). Viable cells which were during September ( $p<0.05$ ). The average number of uric acid crystals was 8.4±5.6/ml. The mean blood cells count, which included Red blood cells, pus cells, epithelial cells and testicular cells, in the semen was 25.2±3x10<sup>3</sup> /ml and it was the highest (29.1±3x10<sup>3</sup> /ml) during August while lowest (22.0±3x10<sup>3</sup> /ml) during November. The average number of faecal droplets in the semen was 14.6±2.1/ml; the highest level of faecal contamination was 17±3/ml in August ( $p<0.05$ ) and lowest level of contamination was 12±2/ml in November ( $p>0.05$ ). Uric acid crystals, faecal droplets and blood cells were contaminants found in almost all the ejaculates but they were in low level. Faecal droplets were relatively in higher proportions in the ejaculates of tolerant and resistant types. Abnormal spermatozoa with coiled, hooked, or ruptured heads and coiled or tailless spermatozoa were found in almost all ejaculates but they accounted only 10% of total cell counts in the samples.

In the total of 509 ejaculates, 57% of donors (290) were found to be submissive to the massage method of semen collection, 28% of donors (142) were tolerant and the remaining 15% of donors (77) were resistant during semen collection (Fig. ??). Pigeons became more and more submissive to the semen collector as they gained experience of handling in course of time.

While examining the colour of cloacal membrane during semen collection, 39% of donors (198) showed reddish cloacal membrane, 43% of donors (219) had pink coloured cloacal membrane and 18% of donors (92) had pale coloured cloacal membrane (Fig. ??).

## 10 IV. Discussion

As in racing pigeons, in the Lahore pigeons also, there is a heavy fluctuation in the semen quality and quantity. Ejaculation performance of domestic pigeons was considerably affected by season, even though pigeons (*Columba livia*) are in fact not typically seasonal breeders (Arieli et al., 1988; Johnston, 1998). Breeding activity of pigeons is high during the spring and summer while low in autumn and winter and hence many birds laid single eggs during the autumn and winter (Riddle, 1971; Janiga, 1985; Janiga and Kocian, 1985), which is mainly due unfavourable weather conditions and shortening of photoperiod that inhibits the gonads development in pigeons (Lofts et al. 1966; Murtonet al. 1973). Similar effect of photoperiod was also demonstrated in Mallards in which increasing photoperiod in the spring and summer has promoted the gonadal growth and increasing plasma testosterone levels (Hasse, 1983). Likewise, most domestic chickens produce high volume of semen during the spring (Saeid and Al-Soudi, 1975). The present study reveals that the ejaculation performance of Lahore pigeons is high during the spring and summer seasons which have characteristic longer photoperiods and low in the autumn and winter seasons which have almost neutral photoperiods under South Indian conditions. The results of present study therefore coincide with the findings of Lofts et al. (1966), Riddle (1971), Murtonet al. (1973), Saeid and Al-Soudi (1975), Hasse (1983), Janiga, (1985), Janiga and Kocian (1985), Johnston (1998) and Cheng et al (2002). For that reason, the major reproductive periods are during these two seasons, even though pigeons will breed throughout the year. After building nests, pigeons perform copulation several times daily, but semen can be obtained hours after first successful ejaculation on the same day. In some cases, pigeons failed to ejaculate and often became aspermic after first success. However, sperm count is restored after at least

2 days of rest from sex. Therefore, daily semen collection would definitely lead to inferior quality semen from pigeons.

According to Owen (1941), the normal semen quantity of household pigeons is 10 -20  $\mu$ L per ejaculate, containing roughly 5-6 million spermatozoa. In racing pigeons the volume of ejaculate is 8.5 -13.5  $\mu$ L (Cheng et al., 2002). In the meantime, in Lahore pigeons the volume of semen per ejaculate is 6.1 -10.8  $\mu$ L, which is comparatively less than the ejaculate of racing pigeons. In general, racing pigeons feed more amounts of food grains than the Lahore pigeons and remain vigorous in the flight activities, so that the semen volume might be higher in comparison to Lahore pigeons. A similar view was also reported by Owen (1941) and Cheng et al (2002). Owen (1941) further concluded that in domestic pigeons, the volume of semen per ejaculate does not appear to be related to the size of birds, which also agrees with the results of present study. But, in chicken and turkey, the volume of semen per ejaculate varies greatly depending on the bird's size and nutrition provided to them because of their large sized internal and external reproductive organs (Lake and Stewart 1978; de Reviers and Williams, 1984), which have variable number of Sertoli cells in testis, that determine the rate of semen production (de Reviers and Williams, 1984).

It is generally accepted that high concentration of spermatozoa is slightly directly proportional to the volume of semen per ejaculate. High volume of semen in the ejaculate contains more amount of seminal plasma while low volume of semen in the ejaculate contains relatively low amount of seminal plasma which is the deciding factor of sperm concentration. All the experimental animals are young adults, semen volume and sperm concentration are always positively correlated ( $r = +0.32$ ;  $p < 0.005$ ). Since the reproductive activity is high during the spring and summer, the sperm concentration is relatively high in these seasons compared to that during the winter and autumn. In this context, the present study is in confirmation with the findings of Murtonet al. (1973), Janiga, (1985), Janiga and Kocian (1985), Johnston (1998) and Cheng et al (2002).

This study makes out a clear point that physiological activities of birds also affect the semen volume per ejaculate and sperm concentration therein. Semen volume and sperm concentration were comparatively low during February, August and September, in which the natural molt occurred in pigeons. At the time of molting, major portion of metabolic energy is diverted to the re-growth of remiges (Decuyper and Verheyen, 1986; Brake, 1993) and decline in the testosterone level in the blood (Zeman et al., 1990), so that there was a marked decrease in semen volume and sperm concentration (Zeman et al., 1990). This decline in testosterone adversely affects the semen quality and reproductive activities (Zeman et al., 1990; Stunden et al., 1998). Similarly, in the male ring dove, semen volume and sperm concentration have been decreasing during the molting period because of reduced level of androgen (Cheng, 1979). Molting is influenced by slightly long photoperiod, health of the bird, decreased plasma thyroxine and increased triiodothyronine (Decuyper and Verheyen, 1986; Brake, 1993). These altered physiological conditions may disturb the reproductive processes, resulting in marked reduction in semen volume and sperm concentration. This study confirms that 3/10 of the male Lahore pigeons experienced a reduced semen volume and sperm count during molting.

Cheng et al (2002) demonstrated that ambient temperature in which pigeons are living affects the ejaculation, semen volume and sperm concentration. Low ambient temperature ( $< 15^{\circ}\text{C}$ ) creates cold stress that forces the bird to reserve energy for maintenance of body temperature, resulting in a lack of sex drive. Similarly, heat stress ( $> 32^{\circ}\text{C}$ ) creates heat induced subfertility, promoting non-viability of sperms (McDaniel et al., 1996), but this condition is reversed when the birds are exposed to suitable temperature range. This rapid response of the domestic fowl to elevated ambient temperatures is not related to impaired sperm formation (McDaniel et al., 1996) because spermatogenesis takes 10 to 12 days followed by 1 to 5 days for sperm to pass through the excurrent ducts (Lake, 1984). However, the ambient temperature has only a negligible effect on the pigeon sperm concentration as recorded by Cheng et al (2002). Thus, thermal stress disturbs semen output rather than production. According to Cheng et al (2002), temperatures between 19 and  $24^{\circ}\text{C}$  are critical for optimal ejaculation performance. Ejaculated spermatozoa of many bird species showed decreased in vitro motility at  $40^{\circ}\text{C}$ , but motility was restored at  $30^{\circ}\text{C}$  (Ashizawa and Sano, 1990; Wishart and Wilson, 1999). Pigeon spermatozoa diluted with Beltsville poultry semen extender exhibited vigorous motility at  $30^{\circ}\text{C}$ , as in other species. The ambient temperature range under Tamilnadu condition is  $21.7 - 32.5^{\circ}\text{C}$  and hence there would be no possibility for inhibition either by heat stress or by cold stress. The ejaculation performance, semen volume and semen count are mainly due to the combined effects of temperature condition and photoperiod.

For breeding purposes, the challenge is to maximize the production of viable or functional spermatozoa per ejaculate (Sexton, 1983). Sexton (1983) and Cheng et al (2002) reported that, for effective fertilization and egg production, the semen should contain 50-80% of viable motile sperms. In the present study, the percentages of motile sperms are within the range of  $70 \pm 2 - 83 \pm 3\%$  and those of viable sperms are within the range of  $61 \pm 3 - 78.5 \pm 3\%$ , which are in the ideal range for maximum reproductive performance of Lahore pigeons.

In addition to sperms, there were uric acid crystals, blood cells, and faecal droplets in the semen. About 6-14 urate crystals were observed per ml of semen, which indicated that uric acid was released during the collection of semen or partly due to retention of a few drops of uric acid in the cloacal pouch before collection. Likewise, 12-16 fecal droplets were noted in every one ml of semen, which denotes that fecal matter comes to the semen either by their release during the collection of semen or by retention of such fecal granules in the cloacal pouch before collection. Ciliates that are the products of rete testis (de Reviers and Williams, 1984) are also found in the ejaculates, but these ciliates do not seem to affect pigeon fertility (Owen, 1941). Blood cells in the semen are

probably the result of rupture of capillaries in the mucosal wall of the cloaca, which are visualized in the semen when it is examined under a microscope. Red blood cells, epithelial cells, testicular cells and pus cells were found in the semen, but Owen (1941) and Chang et al (2002) strongly believe that these cells do not affect the fertility of pigeons. From the present observation, it is clear that such contamination of semen with uric acid, blood cells and fecal droplets is generally low in submissive pigeons, and that semen of tolerant and resistant pigeons is more contaminated with these things. Since, the feces, uric acid and semen are simultaneously discharged through cloacal pouch; such contamination cannot be avoided in birds (Tan, 1980). Semen contaminated with fecal droplets and uric acid is generally not good for artificial insemination because contaminated semen can hamper the fertility in ducks (Tan, 1980). Therefore, it is advised that to avoid contamination with uric acid or feces, the collection frequency should be at least twice weekly and submissive birds should be selected for semen collection (Sexton, 1983;Noirault and Brillard, 1999).

In avian species, ejaculation is initiated by sexual arousal, starting with the ejection of semen from the muscular portion of vas deferens into the urodeum; the erection of fleshy cloacal folds is the sign of a sexually aroused male. Having reached the urodeum, semen gets transported into proctodeum where the edematous phallic folds are visible and mucosal folds are engorged with lymphatic fluid (Lake, 1981;Knight et al., 1984). The filling of the sinus network under the phallic folds resulted in varying degrees of redness of the cloacal mucosa surface, indicating the level of sexual arousal. Sexual arousal is the maximum when the cloacal membrane is red in colour because of passage of more blood to sinus networks in the phallic folds. Sexual arousal is enough for semen collection when the cloacal membrane is pink coloured. A pale membrane indicated that sexual arousal was not sufficiently initiated in these males. Ejaculation rarely occurred when the mucosal membranes were pale, and less semen was ejaculated in an attempt.

The submissive pigeons allow the easy semen collection using manual massage and the tolerant ones let the collectors to collect samples but with little difficulty. On the other hand, the resistant ones get nervousness during semen collection. This phenomenon was more noticeable during the molt stages. Some resistant pigeons became submissive during the successive attempts due to gradual decrease in nervous temperament; this nervous temperament of certain pigeons impaired the sexual arousal, and no ejaculate could be obtained (Lee et al., 1999). These pigeons normally exhibited signs of fear (Craig et al., 1983;Shabalina, 1984;Lee et al., 1999), which is a stress factor associated with poor semen production in roosters pigeons (Lee et al., 1999). In ducks, this stress also inhibited the reflex response to massage (Tan, 1980).

## 11 V. Conclusion

The present study concludes that, though copulation is going on several times every day, manual semen collection can be done twice in a week in pigeons for the purpose of artificial insemination, and that in Lahore pigeons, as in the racing pigeons, ejaculation of semen is high during the spring and summer because of ambient temperature and long photoperiods. Ejaculation performance is high in March and November and low in September. The volume of ejaculated semen, sperm concentration, motile sperms and viable sperms are high during March and November but least in September. Semen qualities such as percentage of motile sperms and viable sperms are between 70 and 86% in the Lahore pigeons, so that semen collected in all the months are ideal for artificial insemination after dilution with Beltsville poultry semen extender. This paper recommends that for breeding purpose semen should be collected from submissive donors which are providing a large volume of semen containing a large proportion of motile and viable sperms.

1

Ingredients	Percentage
Wheat Grains	35 %
Finger Millet	15 %
Pearl Millet	15 %
Green Pea	30 %
Grid*	4.97 %
Vimeral ® **	0.5 ml /
	Pair

\* Grid: I kg contains 100 g charcoal, 100 g egg shell, 75 g limestone, 150 g table salt and 575 g brick powder; \*\* Vimeral ®: 1ml contains vitamin A -12,000 IU; Vitamin B 12 -20 mcg; vitamin D 2 -6,000 IU; and vitamin E -40 mg.

Figure 1: Table 1 :

## 11 V. CONCLUSION

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Month	Average Temperature (°C)	Relative Humidity (%)	Average Photo Period (Hours/Day)	Collecti- At- tempts	Ejaculation (N)	Collection Rate (%)
January	27.0 (24.2-30.2)	68.4-75.0	13.5	80	38	47.6
February	25.6 (21.7-29.2)	62.5-80.4	13.0	70	40	57.1
March	27.5 (25.6-33.1)	63.8-72.4	13.7	80	52	65
April	27.7 (24.7-32.5)	60.5-70.9	14.3	74	46	62.2
May	30.5 (27.2-32.3)	77.6-83.3	15.6	80	45	56.3
June	29.4 (24.4-29.1)	77.1-82.7	15.5	70	41	58.6
July	28.6 (24.6-29.1)	75.1-82.8	15.4	79	46	58.2
August	29.2 (24.5-29.3)	63.5-79.4	14.7	80	37	46.3
September	29.4 (25.1-31.4)	74.0-81.8	14.0	76	34	44.7
October	29.5 (24.5-30.4)	71.1-86.2	13.2	80	36	45.0
November	29.1 (23.7-30.8)	67.9-71.1	12.3	80	51	63.7
December	28.6 (23.4-30.2)	67.1-72.2	12.4	78	43	55.2
Total/Mean $\pm$ SEM				927	509	54.9 $\pm$ 8.8

a denotes the significance ( $P < 0.05$ ) and b denotes the significance ( $P > 0.05$ ).

Figure 2: Table 1 :

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Month	Semen Volume ( $\mu$ l/D/Bird)	Concentration of Sperms (10 <sup>9</sup> /Ml)	Sperm Motility (%)	Sperm Viability (%)	Uric Acid Crystals (No./Ml)	Blood Cells (10 <sup>3</sup> /Ml)	Faecal Droplets (No./Ml)
January	9.2 $\pm$ 0.3 a	4.0 $\pm$ 0.2 a	79 $\pm$ 3 a	78 $\pm$ 6 a	10 $\pm$ 2 a	26.4 $\pm$ 3 a	15 $\pm$ 3 a
February	9.2 $\pm$ 0.2 b	4.1 $\pm$ 0.2 b	79 $\pm$ 2 b	76 $\pm$ 5 a	11 $\pm$ 2 a	25.1 $\pm$ 6 a	16 $\pm$ 3 b
March	10.7 $\pm$ 0.6 b	4.5 $\pm$ 0.3 a	83 $\pm$ 3 a	78 $\pm$ 5 a	14 $\pm$ 2 b	23.1 $\pm$ 2 b	13 $\pm$ 2 a
April	10.4 $\pm$ 0.3 b	4.3 $\pm$ 0.2 b	82 $\pm$ 2 a	76 $\pm$ 6 b	12 $\pm$ 3 b	24.3 $\pm$ 3 b	13 $\pm$ 3 b
May	8.4 $\pm$ 0.3 a	4.1 $\pm$ 0.2 b	79 $\pm$ 2 b	75 $\pm$ 5 b	11 $\pm$ 2 a	26.7 $\pm$ 5 b	14 $\pm$ 2 b
June	10.3 $\pm$ 0.6 a	3.6 $\pm$ 0.3 a	73 $\pm$ 2 b	70 $\pm$ 3 b	8 $\pm$ 2 b	27.4 $\pm$ 6 a	15 $\pm$ 3 a
July	10.8 $\pm$ 0.6 a	3.4 $\pm$ 0.2 a	70 $\pm$ 2 a	68 $\pm$ 5 b	7 $\pm$ 3 a	28.2 $\pm$ 4 b	16 $\pm$ 2 a
August	6.4 $\pm$ 0.3 b	4.1 $\pm$ 0.2 a	68 $\pm$ 3 a	62 $\pm$ 4 a	6 $\pm$ 2 b	29.1 $\pm$ 3 a	17 $\pm$ 3 a
September	6.1 $\pm$ 0.2 b	4.2 $\pm$ 0.1 b	69 $\pm$ 4 b	61 $\pm$ 3 a	5 $\pm$ 2 a	24.3 $\pm$ 4 b	17 $\pm$ 1 b
October	10.2 $\pm$ 0.4 b	3.5 $\pm$ 0.2 a	70 $\pm$ 2 b	63 $\pm$ 3 a	6 $\pm$ 1 b	22.1 $\pm$ 2 a	13 $\pm$ 2 a
November	10.8 $\pm$ 0.7 b	4.5 $\pm$ 0.3 a	83 $\pm$ 3 a	78 $\pm$ 5 b	13 $\pm$ 1 a	22.0 $\pm$ 3 b	12 $\pm$ 2 b
December	9.4 $\pm$ 0.3 a	4.1 $\pm$ 0.2 b	79 $\pm$ 3 b	73 $\pm$ 6 b	10 $\pm$ 1 a	24.2 $\pm$ 5 b	14 $\pm$ 1 b
Total/Mean $\pm$ SEM	9.3 $\pm$ 1.5	4.01 $\pm$ 0.5	76.3 $\pm$ 8	71.8 $\pm$ 10.2	8.4 $\pm$ 5.6	25.2 $\pm$ 3	14.6 $\pm$ 2.1

[Note: a denotes the significance ( $P < 0.05$ ) and b denotes the significance ( $P > 0.05$ ).]

Figure 3: Table 2 :

## .1 Acknowledgements

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