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Research Article

In vitro Storage of Peking Duck Semen in Different Diluents at + 5 °C

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Abstract

In this study, it was aimed to investigate the storage possibilities of Peking duck (*Anas platyrhynchos domesticus*) semen with different diluents at +5 ° C. Semen samples were collected from five male ducks, twice a week for five weeks, using the abdominal massage method. In the research, 50 semen samples were used. Semen from ducks were combined (mixed) after being evaluated individually. Mix volume (ml), density (× 10^9/mL), pH, motility (%) and vitality (%) values were determined as 1.78 ± 0.19, 1.65 ± 0.24, 6.95 ± 0.28, 70.80 ± 2.57, 76.60 ± 2.93 respectively. Glucose (G), Lactated Ringer's (L), Tris (T), Fructose (F), and Dextrose (D) were used as diluents. The reconstituted mixed semen were equilibrated at 37 ° C for 30 minutes and 32-34 ° C for 30 minutes successively and then stored at +5 °C. Average motility (%) values of sperms stored at +5 °C with G, L, T, F and D diluents after 72 hours were found as 41.05 ± 1.79, 9.95 ± 6.50, 2.20 ± 1.13, 28.00 ± 4.15, 24.05±5.85 respectively. Vitality (%) values in the same diluents and under the same conditions were seen as 44.82 ± 1.73, 19.60 ± 9.08, 10.60 ± 4.50, 35.90 ± 4.83 and 33.90 ± 2.68 respectively. In this study, it was determined that the glucose diluent gives positive results compared to other diluents for the storage of peking duck semen at +5 ° C for 72 hours and it can be used for short term storage as a semen diluent in ducks.

Key words: Peking Duck, Semen, Short term storage, Vitality, Motility

Pekin Ördeği Semeninin Farklı Sulandırıcılarda +5 °C' de in vitro Saklanması

Öz

Bu çalışmada; Pekin ördeği (*Anas platyrhynchos domesticus*) semeninin farklı sulandırıcılarla +5°C'de muhafaza edilebilme olanaklarının araştırılması amaçlanmıştır. Semen örnekleri, beş adet erkek ördekten beş hafta süresince haftada iki kez olmak üzere, abdominal masaj yöntemi ile toplanmıştır. Araştırmada 50 semen örneği kullanılmıştır. Ördeklerden alınan semenler bireysel olarak değerlendirildikten sonra birleştirildi (miks yapıldı). Miks semenin volüm (ml), yoğunluk (×10^9 /mL), pH, motilite (%) ve vitalite (%) değerleri sırasıyla 1.78±0.19, 1.65±0.24, 6.95±0.28, 70.80±2.57, 76.60±2.93 olarak tespit edilmiştir. Sulandırıcı olarak; Glikoz (G), Laktatlı Ringer (L), Tris (T), Fruktoz (F), Dekstroz (D) sulandırıcıları kullanılmıştır. Sulandırılan miks semenler sırasıyla 37 °C'de 30 dakika ve 32-34 °C'de 30 dakika ekilibrasyona tabi tutulduktan sonra, +5 °C'de saklanmıştır. G, L, T, F ve D sulandırıcıları ile +5 °C'de saklanan spermaların 72 saat sonra ortalama motilite (%) değerleri sırasıyla 41.05±1.79, 9.95±6.50, 2.20±1.13, 28.00±4.15, 24.05±5.85 olarak bulundu. Aynı sulandırıcılardave aynı şartlarda vitalite (%) değerleri ise; sırasıyla 44.82±1.73, 19.60±9.08, 10.60±4.50, 35.90±4.83 ve 33.90±2.68 olarak tespit edilmiştir.Bu çalışmada pekin ördeği semeninin +5 °C'de 72 saat süreli saklanmasında glikoz sulandırıcısının diğer sulandırıcılara göre iyi sonuç verdiği ve ördeklerde semen sulandırıcısı olarak kısa süreli saklamalarda kullanılabilir olduğu tespit edilmiştir.

Key words: Pekin Ördeği, Semen, Kısa süreli saklama, Vitalite, Motilite

Introduction

Poultry meat and eggs are an indispensable part of our daily diet and a good source of protein (Karakaya and İnci 2014; İnci et al., 2014; Karakaya et al., 2014). Duck husbandry has assumed an important place in poultry

production. There are more than 40 domestic ducks in the world. The most common is the white Beijing duck (Stein, 2012). A total of 524 000 duck are raised for meat, eggs and feathers extensively in the traditionally in Turkey. The White Peking duck is preferred for fairly diseases resistant and a highfeed conversion ratio (FCR) in Turkey (Akpınar et al., 2017).

Artificial insemination is the delivery of spermatozoa taken from the male to the females after being native or diluted, using special tools and techniques. Artificial insemination is the most used, effective and easiest method for breeding breeds in the world. In recent years, artificial insemination has increased in order to reduce operating costs, raise high yield animals, inseminate more females than a male breeding and reduce the risk of venereal (sexually transmitted) diseases. It is very important to collect and store spermatozoa from breeding men to be used in artificial insemination. The purpose of storing spermatozoa collected from breeding men in vitro is to maintain the fertilization ability of spermatozoa. This is only possible when spermatozoa are taken from men by appropriate techniques and stored under appropriate conditions.

Semen is easily taken from the Peking ducks with the method of abdominal massage. However, researches have shown that lymph fluid, blood and feces that come before or after semen during semen collection negatively affect the fertilization ability of duck semen (Fujihara and Mishiyama, 1976; Lake, 1983). In addition, since the frequency of semen collection affects the quality of the semen, the frequency of intake should be limited to twice a week (Tan, 1980; Ghonim et al., 2009).

Spermatozoa in vitro can retain their fertilization abilities only if they are stored under appropriate conditions. Spermatozoa can only retain fertilization abilities for a few hours at room temperature. Extending the fertilization ability of spermatozoa for a few days is possible only by reducing the metabolic rates at low temperatures, meeting the energy and metabolites they need

The process of reducing the metabolic rate of spermatozoa in vitro is provided by lowering the ambient temperature. In order to prevent cold shock effects while lowering the temperature, the temperature drop should be gradual. (eg, 0.5 °C/minute). Also, the temperature should never be lowered below +2 °C to prevent local freezing (Giesen and Sexton, 1983; Blesbois, 2003; Clarke et al., 1982; Wishart, 1984).

The short-term (liquid) storage method of spermatazoa is a method aimed at meeting the energy and metabolites needed by spermatazoa and maintaining their fertility abilities at + 5ºC. Intracellular energy reserves of poultry spermatazoa are very low. The energy and metabolite needs required for spermatozoa to preserve their vitality and activity during in vitro storage must be met from the outside. For this purpose, specially prepared diluents have been developed for poultry spermatozoa. A good diluent; It should be capable of meeting the energy and metabolites required for spermatozoa, protecting them against external factors and not damaging the female genital system. SF, Ringer's, skimmed milk powder, buffer solution and solutions formulated by researchers can be used to store poultry spermatozoa in vitro (Ashizawa and Okauchi, 1984; Blesbois and Caffin, 1992). In a study, it was determined that motility and oxygen consumption were higher in diluents with high seminal plasma amount (Ashizawa and Okauchi, 1984). In addition, dilution rate is very important in the storage process of spermatozoa. This ratio should be (2: 1) 2 parts semen and 1 part diluent. The amount of diluent rate with storage time are directly proportional (Sexton, 1977, 1978 and 1982). In addition to the diluents developed for this purpose, it is necessary to find diluents that can provide low cost, longer duration and higher fertility.

For the determination of fertilization ability and usability of the semen collected from breeding men with appropriate techniques after storage or storage; spermatological values such as amount, motility, vitality, pH and sperm density should be known (Seigneurin and Blesbois, 1995; Alkan et al., 2002). This is possible only with macroscopic and microscopic assays developed for this purpose.

This study was conducted to investigate the effects of different diluents on spermatozoa motility and vitality during short-term storage of duck spermatozoa in vitro and at + 5 ° C.

Material and Method

This work was done in spring to cover April and May. 5 animal male Peking duck (*Anas platyrhynchos domesticus*), 52 weeks old, housed in the application set of Kirsehir Ahi Evran University, Faculty of Agriculture, Animal Science Department was used as animal material. Animal welfare and ethical rules were taken into account in determining the number of animals used in the study. Ducks were not restricted with food and water, were fed with bait as *ad libitum* (18% crude protein and 2300 kcal ME/kg) and natural photoperiod was applied. Ducks were prevented from reaching feed and water 12 hours before the semen was collected to prevent fecal contamination only. All interventions to animals

during the study were carried out in line with the rules reported by Kirsehir Ahi Evran University Animal Experiments Local Ethics Committee and with the approval of the ethical committee numbered AEUHADYEK 01/10 dated 27/01/2016.

Nativ Semen

Collection: Initially, the ducks were accustomed to giving sperm for two weeks. Abdominal massage method was used to collect semen from ducks (Burrows and Quinn, 1937). Semen collection process was carried out in the early hours of the morning, with the aid of mouth large glass tubes, that heated to + 37.5 °C and sterilized. Necessary measures were taken against cold shock and contaminations. This process was continued during five weeks (5 ducks, twice a week, 10 samples from a duck, 50 samples in total).

Macroscopic Inspection: Collected semen were kept at + 37.5 °C until reconstitution. Semen collected before combining were observed separately, care was taken to avoid contamination with feces and blood. 0.01 mL precision injectors (Ayset 70570) were used to determine the amount of semen taken from ducks at once in mL. The collected semen was then mixed (Łukaszewicz et al., 2011; Taskin et al., 2020).

Mix Semen

Spermatozoa Density (ml): It was determined using the hemocytometric method and expressed as $x10^9$ sp/ml. For this purpose, 0.01 ml of sperm was diluted 1/500 with 5 ml of Hayem solution and put into Thoma slide. Sperm counts were performed in 5 large squares in each of the two counting sites on the Thoma slide, and 10 large squares in total. From the values found, the density was calculated with the help of the hemocytometric count equation and expressed as $x10^9$ sp/ml.

$$Density\ (\mu l) = \frac{Number\ of\ Spermatozoa\ Counted}{Large\ Square\ Area\ X\ Large\ Square\ Height\ X\ Reconstitution\ Rate}$$

pH Value: The pH value of the mixed semen was determined with the help of pH 0-14 Universal indicator and expressed as a numerical value (MColorpHast).

Motility (%): Performed at x400 magnification using a phase-contrast microscope (Leica DM750) with a heating table and expressed as %. For this, 5 µl semen was taken on the slide which was heated at + 37.5 ° C and the coverslip was closed at the same temperature. The prepared preparation was placed on a heating plate (Type D, Leica Mats) preheated to + 37F.5 ° C and examined by two observers in at least 3 different microscope fields and expressed as %. In the examination of the mixed semen, it was diluted 1: 1 with physiological serum (PS) in order to better observe motility. In the repeated measurements, no diluent was added (Etches, 1996; Taskin et al., 2020).

***Vitality* (%):** The basis of Eosin nigrosin sperm staining is based on the fact that due to the deterioration of the membrane structure of dead spermatozoa, it takes the dye into the cytoplasm and the sperm appear to be painted. 5 µl of semen was mixed with 10 µl of 1% Eosin-Y in a tube. After 30 seconds, 15 µl of 10% Nigrosin was added and mixed. 1 µl of the mixture was taken and spread on the slide. It was left to dry at room temperature (21 ° C). Then, 200 sperms were examined under a microscope. Dead spermatozoa were observed in red and purple, while living spermatozoa were observed in white and colorless (Lemoine et al., 2011) and expressed as% (Oguntunji et al., 2019; Lemoine et al., 2011; Taskin et al., 2020).

Dilution

The diluents used in the study were prepared as follows;

***Glucose Diluent* (G):** Serum physiological 5% (v/v) 0.37M D (+) - Glucose-monohydrate into

***Lactated Ringer's Diluent* (L):** Lactated Ringer's 100 ml: Sodium laclate: 0.3 g, Sodium chloride: 0.6 g, Potassium Chloride: 0.04 g, Calcium Chloride Dihydrate: 0.03 g, Ejection Water k.m: 100ml, Electrolyte density (MeQ/L): Sodium: 129.3 Potassium: 5.37 Chloride: 112 Calcium: 4 L, Total Osmolar Concentration: 275.52 mOsm /L

***Tris Diluent* (T):** Serum physiological 5% (v/v) 30mM / L Tris (hydroxymethyl)-aminomethane

***Dextrose Diluent* (D):** 5% dextrose, 100 ml: Dextrose (Anhydrus): 5 g, (as Dextrose Monohydrate: 5.5g), Ejection Water k.m: 100ml, Total Osmolar Concentration: 277,47 mOsm/L

***Fructose Diluent* (F):** Serum physiological 5% (v/v) D (-) – Fructose

***Serum Physiological Diluent* (SP):** 9% Isotonic-Sodium Chloride, 100ml: Sodium Chloride: 0.9 g, Water for Ejection k.m: 100ml, Amount of Electrolyte per liter Na^+/154mEq / Cl^-/154 mEq, Total Osmolar Concentration: 308 mOsm/L.

Mix semen, whose spermatological properties were determined, was placed in 0.25 ml of capped and sterile graduated plastic tubes in 5 groups. After the groups formed were numbered, they were diluted 2/1 (2 parts semen, 1 part diluent) using a diluent (G, L, T, F and D) for each group. During this process, care was taken to ensure that the diluents and the sperm were at the same temperature (+ 37.5 °C), and the diluent was added gradually over the sperm.

Storage

The reconstituted mixed semen was equilibrated against cold shock at +37 ° C for 30 minutes and then at +34/32 ° C for 30 minutes and then stored at +5 ° C (Sexton, 1978; Wishart, 1984; Giesen and Sexton, 1983; Blesbois et al., 2006; Taskin et al., 2020). Necessary measures were taken to prevent sudden temperature changes during storage. After the equilibration process, samples were taken for vitality and motlity tests. Then, the semen subjected to equilibration was stored at +5 oC double boiler. Separately from these stored samples, samples were taken for vitality and motility tests at the 5th, 24th, 48th and 72th hours.

Statistical Analysis

The data determined in the study were analyzed using SPSS 15.0 and Windows statistical software program. DUNCAN test was used to analyze one-way analysis of variance and groups. Significance test was made according to P<0.05 and the results were evaluated accordingly.

Results

The daily amount of ejaculate taken from the ducks was determined as average (ml) 0.34 ± 0.05. (Table 1).

Table 1. Daily semen amount

Duck Number	Number (n)	Amount (ml) X̄ ± Sx
1	10	0.35±0.04
2	10	0.36±0.05
3	10	0.33±0.04
4	10	0.35±0.04
5	10	0.33±0.06
Total	50	0.34±0.05

The average amount of mixed semen is 1.76 ± 0.16 ml, pH value is 6.95 ± 0.28, density is 1.65 ± 0.24x109/ml, motility is 70.80% ± 2.57 and vitality is 76.60% ± 2.93 detected to be (Table 2).

The motility values of mixed semen diluted with G, L, T, F and D at the end of equilibration are 63.85 ± 4.91, 60.35 ± 5.70, 56.10 ± 5.34, 63.95 ± 7.18 and 64.95 ± 3.75 respectively. At the end of the 72 hours, 41.05 ± 1.79, 9.95 ± 6.50, 2.20 ± 1.13, 28.00 ± 4.15 and 24.05 ± 5.85 was determined to be (Table 3).

Table 2. Spermatological value table of mixed semen

Spermological characteristics	(X̄ ± Sx)
Daily Amount (mL)	1.76±0.16
pH	6.95±0.28
Density (x10^9 /ml)	1.65±0.24
Motility (%)	70.80±2.57
Vitality (%)	76.60±2.93

Table 3. Time-dependent motility (%) change table in G,L, T, F, D diluents (P<0.05)

Diluent	(G) X̄± Sx	(L) X̄± Sx	(T) X̄± Sx	(F) X̄± Sx	(D) X̄± Sx
Equilibration	63.85±4.91^a	60.35±5.70^a	56.10±5.34^b	63.95±7.18^a	64.95±3.75^a
5 hours	62.80±3.48^a	56.40±4.76^b	43.15±5.38^c	56.65±7.10^b	57.60±3.96^b
24 hours	55.70±3.68^a	44.00±9.90^c	26.35±5.00^d	52.83±5.07^{ab}	50.00±3.98^b
48 hours	50.60±2.54^a	28.30±8.99^c	10.30±2.37^d	42.80±3.48^b	41.35±5.01^b
72 hours	41.05±1.79^a	9.95±6.50^c	2.20±1.13^d	28.00±4.15^b	24.05±5.85^b

Eosin nigrosin sperm staining technique was use to determine the vitality (%) of mixed semen. Dead spermatozoa were observed in red, purple, and live spermatozoa were white, colorless (Figure 1)..

Figure 1. Microscopic view of spermatozoa (A) dead, (B) live (Leica DM750, x400)

If the vitality (%) value of the mixed semen is; at G, L, T, F and D, the end of equilibration is 72.05 ± 4.10, 70.35 ± 5.70, 63.60 ± 6.57, 70.10 ± 6.23, 71.40 ± 5.27 respectively. At the end of the 72 hours 44.82 ± 1.73, 19.60 ± 9.08, 10.60 ± 4.50, 35.90 ± 4.83 and 33.90 ± 2.68 was determined as (Table 4).

Table 4. Time-dependent vitality (%) change table in G, L, T, F, D diluents (P<0.05)

Diluent	(G) X̄± Sx	(L) X̄± Sx	(T) X̄± Sx	(F) X̄± Sx	(D) X̄± Sx
Equilibration	72.05±4.10[a]	70.35±5.70[b]	63.60±6.57[c]	70.10±6.23[b]	71.40±5.27[b]
5 hours	67.60±4.38[a]	63.3±5.67[a]	54.30±7.20[b]	66.55±6.78[a]	64.00±6.01[a]
24 hours	61.25±3.12[a]	52.50±10.06[b]	45.50±3.92[c]	62.60±4.46[a]	59.05±4.07[a]
48 hours	53.75±2.17[ab]	39.75±10.40[c]	21.40±3.51[d]	57.20±6.19[a]	50.95±5.07[b]
72 hours	44.82±1.73[a]	19.60±9.08[c]	10.60±4.50[d]	35.90±4.83[b]	33.90±2.68[b]

Discussion

In our study, the average daily amount of ejaculate taken from ducks at one time was determined to be 0.34 ± 0.505 ml. Similarly, Nahak et al. (2015) determined this value as an average of 0.74 ± 0.07 ml from 35-40 week old ducks. In contrast, in a study by Zawadzka and et al. (2015), they were determined to be 0.23 ± 0.02 ml in 52-week-old ducks. The value we obtained is similar to the value obtained by J. Zawadzka et al. (2015). As a reason for this, it is thought that the age of the animal material used in the study is close, and the frequency of ejaculate intake and can also be effective of season.

The average pH of the mixed semen was measured as 6.95 ± 0.28. This value that we found is quite close to the pH value determined by J. Zawadzka et al., (2015) as 6.90 ± 0.44 in their studies. In addition, pH value in our study is similar to 7.51 ± 0.21 (Wagner and Pingel, 1995) and 7.48 ± 0.06 (Cyriac et al., 2013) determined in the

literature. In addition, it has been reported that semen pH is influenced by density and measuring time (Bandyopadhyaya et al., 2007).

Surai and Wishart (1996) determined semen density as 1.5-8.0 x109/ml, while Nahak et al. (2015) determined 0.84-1.3x109/ml. The density value of 1.65 ± 0.24 × 109/ml we found in our study is similar to the value of Surai and Wishart (1996). It has been reported that the effect of frequency of sperm intake on semen density is similar to the effect on quantity (Zawadzka, et al., 2015). In addition, it has been reported in a study that vitamin E and selenium increase the density and amount of duck semen (Safaa et al., 2019).

The motility and vitality values of the mixed semen before reconstitution were determined 70.70 % ± 2.57 and 76.60 % ± 2.93, respectively. In similar studies, the motility was found 72.85 %, 61.1 % and 60.83 %, while the vitality value was faund at 67.90 % (Kontecka, 1992; Kasai and Izumo, 2001; Cyriac et al.,2013).

In this study, spermatazoa stored in five different diluents for 72 hours at + 5 ° C were evaluated in terms of motility values (Table 3). In this process, the difference between T diluent and other diluents was found significant at the end of equilibration (P <0.05) and the highest motility value was determined in diluent D with 64.95 % ± 3.75. At the end of the 5th hour, the motility results of L, F, D diluents were found similar, but the differences between them and other diluents were found to be important. The highest motility value was determined at G and the lowest at T (P <0.05). F and G and D diluents were similar at the end of 24 hours. The highest motility value was determined in G diluent with 55.70 % ± 3.68 (P <0.01). At the end of the 48th hour, similarities were observed between the F and G diluents, and the differences between the diluents were found significant at the level of P <0.01. The highest motility value was determined in the G diluent with 50.60 %± 2.54. At the end of the 72th hour, similarities were observed between the F and G diluents, and the differences between the diluents were found significant at the level of P <0.01. The highest motility value was determined in G diluent with 41.05% ± 1.79, and the lowest motility value was found in T diluent with 2.20 %± 1.13.

Also in this study, time-dependent (Equilibration, 5th, 24th, 48th and 72th hour) changes in vitality were detected (Table 3). L, F, D similarity was observed between diluents in terms of vitality at the end of equilibration, the difference between other diluents was found to be significant at P <0.05 level and the highest vitality value was determined in G diluent with 72.05 ± 4.10. At the end of the 5th hour, the difference between the T diluent and other diluents was found to be significant at the level of P <0.05 and the highest vitality value was found in the G diluent with 67.60 ± 4.38. At the end of the 24th hour, similarities were observed between the G, F and D diluents, and the difference between the diluents was found to be significant at the level of P <0.01. The lowest vitality value was determined in the T diluent with 45.50 ± 3.92. At the end of the 48th hour, the highest vitality value was determined in the F diluent with 57.20 ± 6.19 (P <0.01). At the end of the 72th hour, while F and D formed a group, the highest vitality value was determined in the G diluent with 44.82 ± 1.73, and the lowest motility value was found in the T diluent with 10.60 ± 4.50 (P <0.05).

It has been determined that the differences in the motility and vitality value of spermatazoa stored in five different diluents for 72 hours at +5 °C are due to the chemical structure and density of the diluents. Lake and Ravie (1987) found similar results to our results in their study.

As a result, in this study it was determined that the glucose diluent (G) had a positive effect on the motility and vitality values of duck spermatazoa that were stored at + 5 ° C for 72 hours compared to other diluents..

Conflict of Interest Statement: The manuscript's authors declare that, they do not have any conflict of interest.

Researchers' Contribution Rate Statement Summary: The authors declare that, they have contributed equally to the manuscript.

References

Akpınar, G.Ç., Alaşahan, S.S. and Canoğulları, D. 2017. Halk elinde yetiştirilen Pekin ördeklerinde matematiksel formüller ile yumurta kalite özelliklerinin belirlenmesi openaccess.mku.edu.tr.

Alkan, S., Baran A., Özdaş, B. and Evecen, M. 2002. Morphological defects in turkey semen. Turk. J. Vet. Anim. Sci. 26, 1087-1092.

Ashizawa, K. and Okauchi, K. 1984. Stimulation of sperm motility and oxygen consuption f fowl spermatozoa by a low molecular weight fraction of seminal plasma. J. Reprod. Fertil. 71(2), 593-598.

Bandyopadhyaya, S.K., Bhatacharya, Choudhury RR, Basu S. 2007. Text book of gynaecology, artificial insemination, obstetrics and assisted reproduction. 2nd ed. India: Kalyani Publishers/Lyall Bk Depot.

Blesbois, E. 2003. Semen storage in turkeys: current status and future practice. In Fifth international symposium on turkey reproduction. Raleigh, USA. pp. 96–100.

Blesbois, E. and Caffin, J.P. 1992. "Serum Like" Albumin of fowl seminal plasma and effects of albumin on fowl spermatozoa stored at 4°C. Br. Poult. Sci. 33(3), 663-670.

Blesbois, E., Dubos, F., Grasseau, I., Richard, M.M., Roman, Y., Saint, J.M. and Seigneurin, F. 2006. Cryopreservation of avian spermatozoa and predictors of ability to freezing. Les Actes du BRG 6, 415–431.

Clarke, R.N., Sexton, T.J. and Ottinger, M.A. 1982. Effects of holding temperature on storage time, on respiratory rate, motility and fertility of chicken and turkey semen. Poult. Sci. 61, 1912-1917.

Cyriac, S., Joseph, L., Peethambaran, P.A., Narayanankutty, K. and Karthıayını, K. 2013. Semen quality characteristics of White Pekin, Kuttanad (Anas platyrhynchos domesticus) and Muscovy (Cairina moschata momelanotus) drakes. Indian J. Anim. Sci. 83(6), 595-599.

Etches, R. J. (1996). Artificial insemination. In: Reprod poult. Cambridge. Wallingford: CAB International, 234-262.

Fujihara, N. and Nishiyama, H. 1976. Studies on the accessory reproductive organs in the drake. 5. Effects of the fluid from the ejaculatory groove on the spermatozoa of the drake. Poult Sci. 55, 2415-2417

Ghonim, A.I.A., Awad, A.L., El-Sawy, M.A., Fatouh, M.H. and Zenat, A.I. 2009. Effect of frequency of semen collection, dilution rate and insemination dose on semen characteristics and fertility of Domyati ducks. Egypt. Poult. Sci. J. 29, 1023-1045.

Giesen, A.F. and Sexton, T.J. 1983. Beltsville poultry semen extender. 9. Effect of storage temperature on turkey semen held eighteen hours. Poult Sci. 62(7), 1305-1311.

İnci, H., Karakaya, E., Şengül, T., Söğüt, B., 2014. Bingöl ilinde kanatlı eti tüketiminin yapısı. Türk Tarım ve Doğa Bilimleri Dergisi 1(1): 17- 24.

Karakaya, E., İnci, H., 2014. Bingöl ili merkez ilçesi hane halkının kanatlı eti tüketim tercihleri. Uludağ Üniversitesi Ziraat Fakültesi Dergisi 28 (1), 53-64.

Karakaya, E., İnci, H., Söğüt, B., Şengül, T. 2014. Bingöl İl Merkezinde Yaşayan Hane Halklarının Yumurta Tüketim Durumu Üzerine Bir Araştırma, Türk Tarım ve Doğa Bilimleri Dergisi, 1(2): 239-247.

Kasai, K. and Izumo, A. 2001. Efficiency of artificial vagina method in semen collection from Osaka Drakes. J. App. Poult. Res. 10, 206-210.

Kontecka, H. 1992. Sperm quality changes in drakes during the reproductive season. Rocz. Nauk. Zoot. 1 (19), 9-18.

Lake, P.E. 1983. The male in reproduction. In: Physiology and Biochemistry of the domestic fowl. Ed: B. K. Freeman. 5, 1-61.

Lake, P.E. and Ravie, O. 1987. Effect on fertility of low numbers of fowl spermatozoa İnseminated in aqueous dilucnt or scmcn components of the fowl and turkey. Br. Poult. Sci., 28, 75-80.

Lemoine, M., Mignon, S., Grasseau, I., Magistrini, M. and Bleisbois, E. 2011. Ability of chicken spermatozoa to undergo acrosome reaction after liquid storage or cryopreservation. Theriogenology 75, 122-130.

Lemoine, M., Mignon-Grasteau, S., Grasseau, I., Magistrini, M., Blesbois, E. 2011. Ability of chicken spermatozoa to undergo acrosome reaction after liquid storage or cryopreservation. Theriogenology, 751: 122-130.

Łukaszewicz, E., A, Kowalczyk., Zońca, Z.R., 2011: Characteristics of fresh semen of captive-bred Capercaillie Tetrao urogallus L. Zoo Biol. 30, 672-680.

Nahak, A.K., Giri, S.C., Mohanty, D.N., Mishra, P.C. and Dash, S.K. 2015. Effect of frequency of collection on seminal characteristics of White Pekin duck. Asian Pacific Journal of Reproduction; 4(1), 70-73.

Oguntunji, A. O., Oladejo, A. O., Ayoola, M. O., Oriye, L. O. and Egunjobi, I. M. (2019). Semen Quality Parameters of Three Duck Genotypes in the Humid Tropics Zhivotnovadni Nauki, 56(4), 50-58.

Safaa, A.M., Elsyed I.E., Hassan, A. and Hassan, A.M. (2019). Effect of vitamin e-selenium supplementation on some semen quality traits of muscovy drake. Arab Univ. J. Agric. Sci., 27(2),1627-1636.

Seigneurin, F. and Blesbois, E. 1995. Effects of the freezing rate on viability and fertility of frozen-thawed fowl spermatozoa. Theriogenology 43, 1351-1358.

Sexton, T.J. 1977. A new poultry semen extender. 1. Effect of extension on the fertility of chicken semen. Poult. Sci. 56, 1443-1446.

Sexton, T.J. 1978. A new poultry extender. 3. Effect of storage conditions on the fertility capacity of chicken semen stored at 5°C. Poult. Sci. 57, 285-289.

Sexton, T.J. 1982. Beltsville poultry semen extender. 6. Holding turkey semen for six hours at 15°C. Poult. Sci. 61, 1202-1208.

Stein, B. 2012. Introduction to commercial duck farming. NSW Government, Department of primary industries, Goulburn. http://www.dpi.nsw.gov.au/ data/ assets/ pdf file/ 0009/442854/ introduction-tocommercial-duck-farming.pdf (Date of Access: 24.02.2020).

Suraı, P.F., Wıshart, G.J. 1996. Poultry artificial insemination technology in the countries of the former USSR. World Poult. Sci. J., 52(1). 27-43

Tan, N.S. 1980. The frequency of collection and semen production in Muscovy drakes. Br. Poult. Sci. 21, 265-272.

Taskin, A., Ergün, F., Karadavut, U., Ergün, D. 2020. Effects of extenders and cryoprotectants on cryopreservation of duck semen. Turkish Journal of Agriculture - Food Science and Technology, 8(9): 1965-1970.

Wagner, A. and Pıngel H. 1995. Characteristics of semen of Muscovy and Pekin drakes. Proc. 10th Eur. Symp. Waterfowl, Halle, Germany, 286-290.

Wishart, G. 1984. Metabolism of fowl and turkey spermatozoa at low temperatures. J. Reprod. Fertil. 70, 145-149.

Zawadzka, J., Łukaszewicz, E. and Kowalczyk, A. 2015. Comparative semen analysis of two Polish duck strains from a conservation programme. Europ. Poult. Sci., 79, 1-9.