EFFECT OF ADDITION FROM EGG YOLK OF DIFFERENT AVIAN TO TRIS EXTENDER ON FREEZING SEMEN TRAITS OF AWASSI RAMS

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ABSTRACT

The objective of this study was to investiget the effect of different kinds of egg yolk (hen, quail, turkey, and duck) on the awassi ram semen quality after cryopreservation. The semen was collected by using artificial vagina from four awassi rams and then evaluated , after pooling the semen samples and dilution with tris which contain egg yolk made from poultry birds (10%) and glycerol (5%).The samples were kept in 4C for two hours and then exposed to liquid nitrogen vapor for (10min) and suck in liquid nitrogen, one month later, the samples were thawed at 37C for(5min).the sperm motility, membrane integrity, the percentage of dead and abnormal sperm and DNA fragmentation determination were evaluated. The results showed that yolk of quail egg recorded the higher percent of sperm motility and membrane integrity in comparison with other birds (40.85, 43.85%) respectively. No significant (P>0.05) differences were recorded between quail yolk egg and duck yolk egg for dead and abnormal sperm. The yolk of quail eggs Foard the lowest percentage of DNA fragmentation (8.8%) while no significant difference was recorded between yolk of hen egg, turkey and duck. It can be conclude that the ability of using yolk of quail eggs as substitute of yolk of hen in the semen ram dilutions.

Key Words: semen, yolk Sac, cryopreservation

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تأثير اضافة صفار البيض لطيور من مصدر مختلفة على صفات السائل المنوي للكباش العواسي

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المستخلص

كان الهدف من هذه الدراسة هو تحديد تأثير أنواع مختلفة من صفار البيض (الدجاج،السمان،التركي،البط) على نوعية السائل المنوي للكباش العواسية بعد الحفظ بالتجميد.تم جمع السائل المنوي باستخدام المهبل الاصطناعي من أربع كباش عواسي وتقيمه ، وبعد تجميع عينات السائل المنوي ،واجراء التخفيف بمخفف الترس والذي يحتوي على صفار البيض لطيور الداجنة (10٪) وكليسيرول (5٪). تم وضع العينات عند درجة حرارة 4 ْم لمدة ساعتين، بعدها تعرضت العينات لبخار النيتروجين السائل لمدة (10 دقائق) وتغطس في النيتروجين السائل، بعد شهر يتمت أذابة العينات عند درجة حرارة 37ْم لمدة 5 دقائق،تم تقييم حركة وسلامة الغشاء البلازمي للنطف ، ونطف الميتة والمشوهة وتجزؤ المادة الوراثية. أظهرت النتائج ان صفار بيض السمان سجل اعلى نسبة مئوية لحركة وسلامة الغشاء البلازمي بالمقارنة مع صفار بيض الطيور الاخرى(40.85 ،43.85%)بالترتيب .لم تسجل فروقات معنوية (P>0.05) بين صفار بيض السمان والبط للنطف الميتة والمشوهة ، سجل صفار بيض السمان اقل نسبة مئوية لتجزؤ المادة الوراثية (8.8%) في حين لم تظهر فروقات معنويه بين صفار بيض الدجاج والتركي والبط. تشير النتائج الى امكانية أستعمال صفار بيض السمان كبديل عن صفار بيض الدجاج في مخفف السائل المنوي للكباش.

الكلمات المفتاحية: السائل المنوي , صفار البيض , التجميد

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INTRODUCTION

For obtaining the best results from the use of artificial insemination in farm animals, especially in small ruminants is the use of males with excellent genetic characteristics thus spread these qualities to the rest of the animals and thus ensure the best results (2) The importance of using methods that maintain a quality and to contribute to the protection of the fertilization capacity of the sperm without damage. These are important conditions that must be available when semen collection and storage for fruition goals of artificial insemination (6). The discovery of Philips significantly of adding egg yolk to the extenders of semen intended to be an important step in the artificial insemination development (25). Egg yolk is an important component of the material that contributes in effectively to the sperm preservation. The most important of these substances are lipoproteins and lysithins which have a great role in preserving the sperm from cold shock during. Processes of cooling or freezing. The Researchers also, contribute effectively to maintaining the osmotic pressure during the semen dilution processes (27). That is the role of low-density lipoproteins by stabilizing the sperm membranes and replacing the phosphoproteins found in the sperm membranes that are damaged during conservation (24, 4). The aim of this study was to compare the egg yolk optioned from different avian (Chicken, quail, turkey, ducks) and there's effect on the seminal fluid characteristics of the rams after the cryopreservation . up to make a pseudo mount to increase their sexual Libido (3).

MATERIALS AND METHODS

Semen Collocation This study was carriedout at the College of Agriculture, University of Baghdad, in cooperation with the Higher Institute of Infertility and Reproductive Assisting Techno-logy, Nahrain University, and the Department of Agricultural Research / Ministry of Agriculture, during May to October 2015, using 4 rams, at 8 am, with a ejaculate ram / week, and artificial vagina for sheep and goats. The rams are set .

Semen dilatation

The samples of semen were diluted with Tris extender and the dilute was prepared according to the method described by Evans and Maxwell (11) and the egg yolk (10%) v/v Penicillin and streptomycin were enriched to the extender (100.000 IU and 100 mg, respectively).was added to four poultry (chicken, quail, duck, turkey) and glycerol (5%) v/v. After completion of the collection and sample tests, the additive is added by a pipette inserted into the semen gradually and not the other way around to avoid a shock to the sperm as the sample is diluted 1: 1 with the Tris. The sperm samples are collected and the dilution is completed 10: 1, and at 5 ° C, dilution 20: 1, which contains 5% capsule per 100 ml of dilute, and is left for two hours to be an equilibrium period as indicated (12).

Freezing technique

After collecting the semen and conducting the tests for evaluation and mitigation , then save it in a heat-insulating container and transfer it to the Institute for the diagnosis of infertility and assisted reproductive technologies / University of Nahrain, and then fill the sperm and placed in the special carrier of Cryovial, and leave at 5 m for an hour then Samples of nitrogen vapor are subjected to 10 minutes of nitrogen vapor (-75° C) and then immersed in nitrogen (-196° C) and left for one month. Cryovial is placed in the water bath at 37 ° C for 5 minutes.

Evaluation of semen

Sperm motility The individual motility of the sperm after thawing was estimated by placing a diameter of dissolved semen on a warm slice at a temperature of 37 ° C and measured at a magnification of 400x (7).

Sperm mortality and abnormality

The percentage of dead sperm was estimated based on the results of Swanson and Beardon (29). The same percentage of the dead sperm was estimated as the percentage of deformed sperm, according to Hancock (14).

Sperm membrane integrity test

This test is called a water test. The percentage of safety of the plasma membrane is estimated based on what it says Lomeo and Giambersio. (22).

Sperm DNA fragmentation

The alcidine orange stain was used as stated by Tejada , Mitchell and Norman (30).

Statistical analyses : The data were statistically analyzed using SPSS/PC version

18 software (SPSS, Chicago). Sperm parameters were analyzed using complete randomized design (CRD). The Statistical model was

Yij = μ + Ti + eij.

Where

Yij= dependent variables sperm parameters

μ= overall mean. Ti= effect of treatments (addition of egg yolk). eij= error term. Differences among means were computed using the Duncan Multiple Ranges Test Duncan (9).

RESULTE AND DISCUSSION

The semen quality deterioration is often due to cold shock at 5 ° C, which affects the sperm metabolic capacity due to the release of enzymes and ions (8) and (23). The cryopreservation process, including the cooling process, leads to mechanical stress in sperm plasma membrane and lost of osmotic system that occur to change in entry of water to the sperm (13). The egg yolk is a common element in most of the thinners used for the freezing of semen in farm animals for a cycle in the protection of the plasma membrane and the sperm acrosome, It is believed that phospholipids, cholesterol and low-density lipoproteins are the most important factors that preserve the sperm from the changes taking place during freezing phases (1). The results of this study showed the effect of the addition of egg yolk for different types of avian, (quail, duck, turkey and chicken) to dilute the cryopreserved semen. The results revealed the individual sperm motility significant increase ($P\leq 0.05$) when addition Quail egg yolk comparing with the others treatments it was 40.85% (Figure 1). These results were differed from those of El-Badry**,** et al (10), they pointed out that the addition of Turky egg yolk gave the best results for the sperm motility compared to the addition of egg yolks, Quail, chicken and ducks to the frozen buffalo sperm, as well as the percentage of pigs (18) and semen frozen in dogs (4) While the results of Rauch (26) approval of the results of this study, where he explained that adding the yolk of the quail to the frozen sperm of the bulls contributed to improve the sperm motility compared to the chicken eggs while Kulaksiz , Cebi ,Akcay and Daskin (20) Found the addition of the yolk of the quail eggs to the frozen sperm of the bulls did not give significant differences in the sperm motility after addition, Chicken egg yolk. The results of this study also showed a significant superiority ($P< 0.05$) for the treatment of the addition of egg yolks compared to the other treatments it was (43.85) (Figure2). While El-Badry,Rawash, Manal Abd Elaal and Amal (10) found the use of egg yolk for different types of birds did not significant differences in plasma membrane integrity ratio in frozen sperm of buffalo bulls. From Figure 3 shows the results of the percentage of sperm mortality, where the treatment of adding chicken yolks recorded the highest percentage of sperm mortality and significant increase ($P< 0.05$) compared to the other of the treatments. The results in the Figure 4 and Figure 5 .are show the percentage of sperm abnormality and DNA fragmentation The addition of chicken egg yolk was significantly higher ($P< 0.05$) when , compared to other treatments. Kampshmidt, et al (19) conduced that the presence of granules in the egg yolk of all species of avian works to reduce the motility of the sperm and the presence of progesterone in the egg yolk works to inhibit the capacitating of the uterus and Van Wagtendonk et al (31) that egg yolk is a source of bacterial contamination. The results of this study was indicated that the addition of egg yolks to quenching of sperm for cryopreserved sheep gave the best results for the motility of the sperm, the integrity of the plasma membrane, the breakdown of DNA and the dead and sperm abnormality of the egg yolk (depends on the containment of cholesterol, phosphorus and low density lipoprotein).Ice and ice crystals within cells, thereby protecting the safety of sperm plasma membranes against cold shock during freezing and thawing (15). The addition of egg yolk to stallion sperm diluents significantly improved plasma membrane integrity and DNA integrity 2.0 %(28, 29), 4.0% (30), 20.0% (30, 31) It can be conclude that the ability of using yolk of quail eggs as substitute of yolk of hen in the semen ram dilutions.

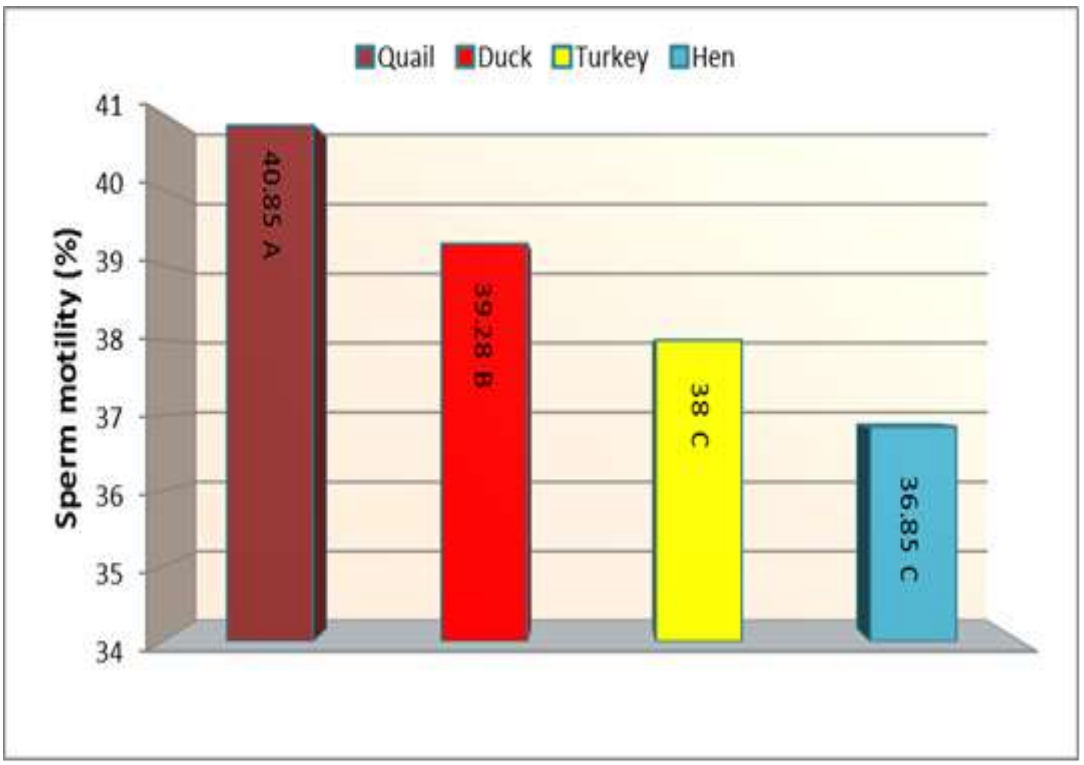

Figure 1. Effect of addition of egg yolk for different types of avian to extender of semen on the percentage of sperm motility

Columns with different letters were significant differences (P< 0.05).
Columns with similar letters with no significant differences (P >0.05).-

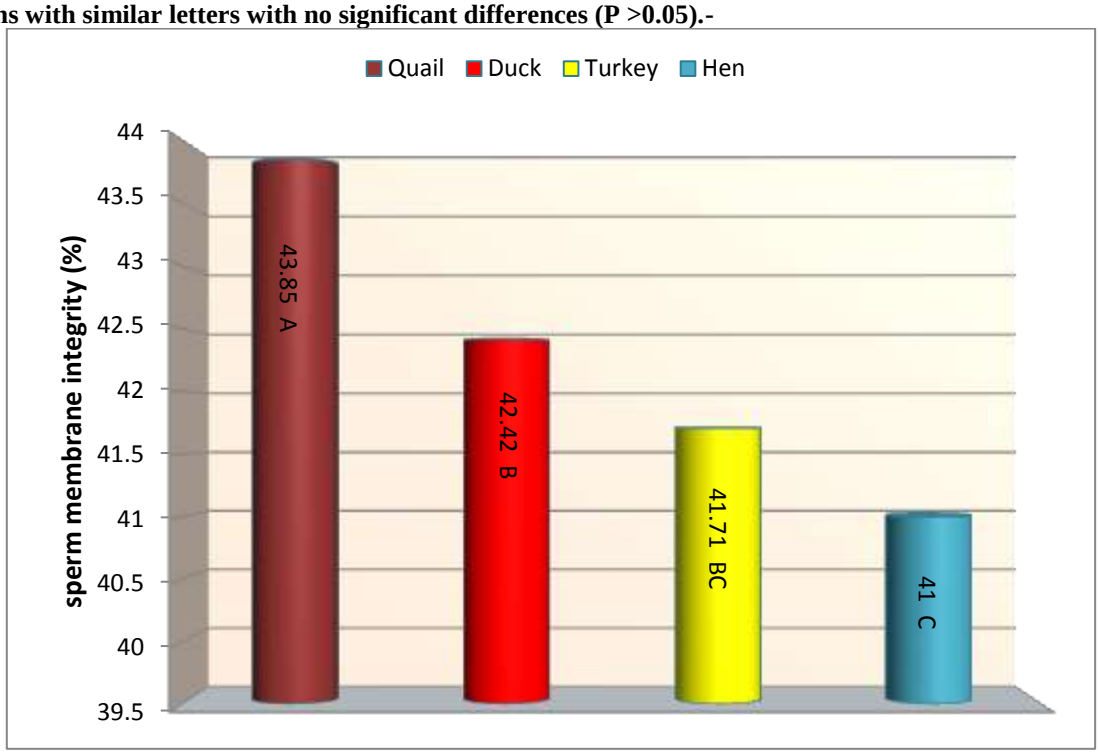

Figure 2.Effect of addition of egg yolk for different types of avian to extender of semen on the percentage of sperm membrane integrity.

Columns with different letters were significant differences (P< 0.05).
Columns with similar letters with no significant differences (P >0.05).

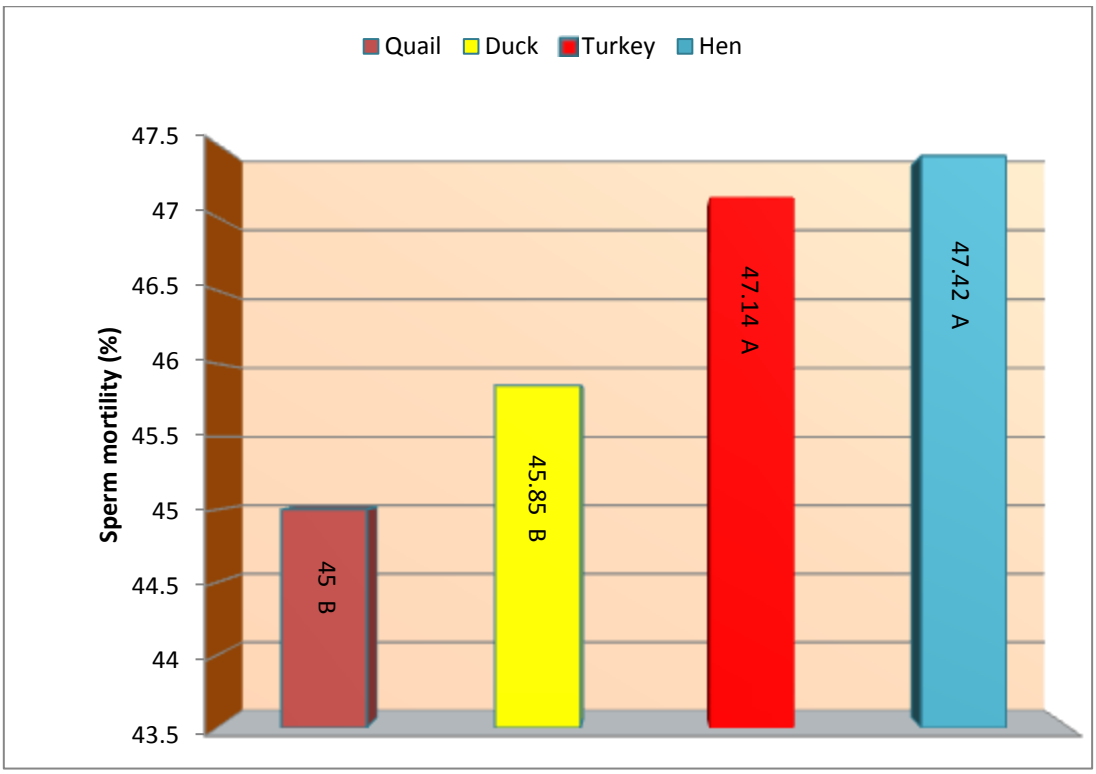

Figure 3. Effect of addition of egg yolk for different types of avian to extender of semen on the percentage of sperm mortality

Columns with different letters were significant differences (P< 0.05) .
Columns with similar letters with no significant differences (P >0.05).

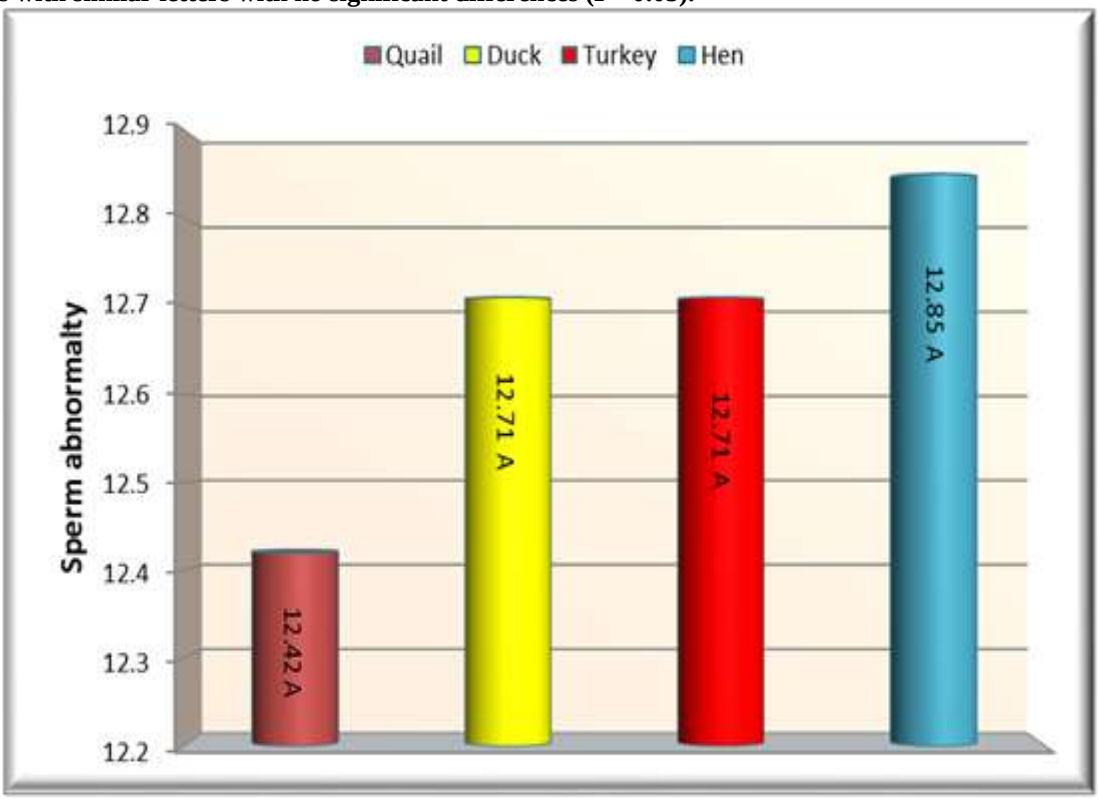

Figure 4. Effect of addition of egg yolk for different types of avian to extender of semen on the percentage of sperm abnormality

Columns with different letters were significant differences (P< 0.05).
Columns with similar letters with no significant differences (P >0.05).-

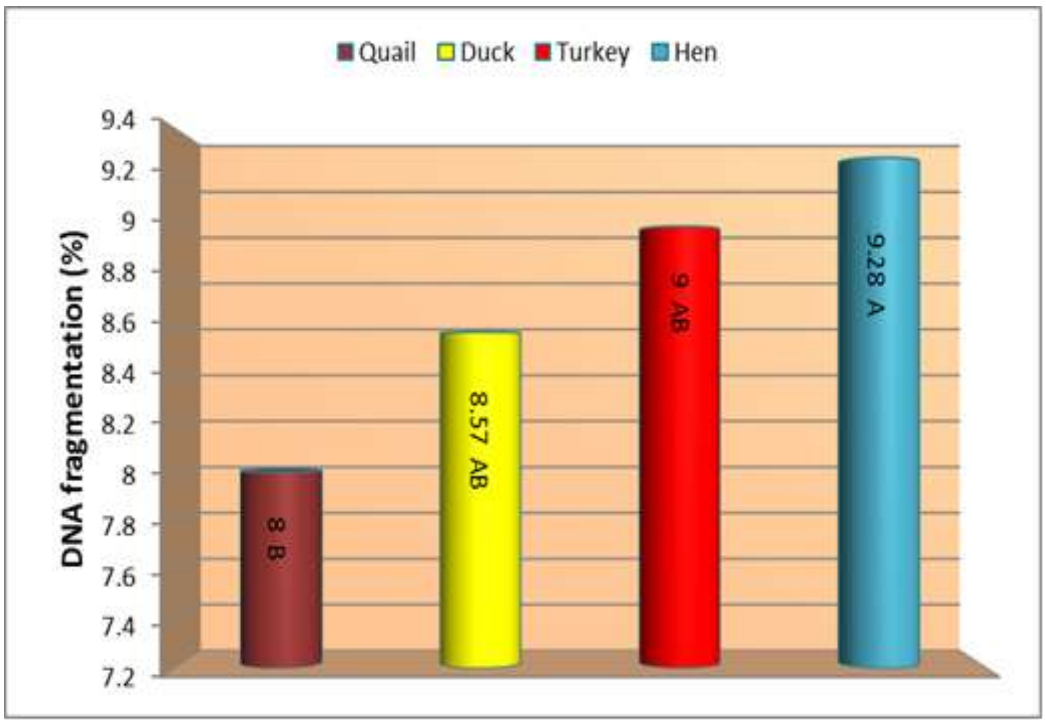

Figure 5. Effect of addition of egg yolk for different types of avian to extender of semen on the percentage of sperm DNA fragmentation.

Columns with different letters were significant differences (P< 0.05) .
Columns with similar letters with no significant differences (P >0.05)

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