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# Comparative Study of Using Four Types of Avian Egg Yolk on Epididymal Sperms Chilled Storage in Awassi Rams *(Ovis aries)*

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## ABSTRACT

The objective of the current experiment was to study the effect of different avian egg yolks (EYs) on rams epididymal sperms stored at 4 ºC. Sixteen pairs of testes (n=32) were mature Awassi rams of about 1-4 years, slaughtered at Al-Sadoon abattoir, Mosul city were collected. The spermatozoa were collected from the tail of the epididymis by cutting and squeezing; after that, a total of 0.5 ml of spermatozoa volume was diluted by using sodium citrate 2.9% and the volume was completed to 1 ml, then the sample was divided into four aliquots (0.25 ml for each one). The extender consisted of egg yolk (EY) 10% of four avian types (chicken, quail, duck, turkey), sodium citrate 2.9%, fructose 2.4gm, penicillin, and streptomycin (100.000 IU and 100 mg, respectively) in 100 distal water. Sperms individual motility, live sperm percentage, sperms abnormalities were checked at 0, 72, 144 hours of storage. Results of the present study revealed that quail egg yolk improved sperms, individual motility, live sperm percentage with longer life span and reduce sperms abnormalities, followed by Turkey, chicken and duck EYs, respectively. Individual motility of the spermatozoa after 144 h of storage in quail EY extender were (36.00%± 1.20), which higher than turkey EY (32.30%± 1.30). However, these results were higher and significant (P< 0.05) when compared with chicken EY extender (24.60%± 2.30) and duck EY extender (19.00%± 1.50). Similar results of quail and turkey EYs were manifested in the percentage of live spermatozoa, which were (38.00%± 1.20), (33.30%± 1.30), (24.00%± 2.30), and (19.90%± 1.50), respectively. The duck EY was significantly lower (P< 0.05) when comparing the four egg yolks types after storage for 144 h. In conclusion: we advise potentially using a quail or turkey EYs extender for the chilled storage for ram epididymal sperms.

**Keywords**: Avian egg yolk, Epididymal sperm, Ram, Semen.

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## INTRODUCTION

In different animals and ruminants, Cauda epididymal sperm can be considered the only available source of male gametes for use in assisted reproductive programs (**Mahdi *et al.*, 2019**). The cauda epididymis sperms could be recovered and stored in the same way as ejaculated spermatozoa (**Abu *et al.*, 2016; Tunner *et al.*, 2020**). Demanding epididymal sperms at post mortem is essential in cases such as the unexpected death of genetically invaluable livestock males and endangered wild species (**Ahmadi *et al.*, 2020**). Egg yolk (EY) is a unique component most widely used for semen storage, manipulation, dilution and preservation due to an extensive variety of elements that supported its effect on spermatozoal movement, viability and freezing (**Al-any *et al.*, 2016**). However, its composition and content of Low-Density Lipoprotein (LDL), biochemical compounds, vitamins and unsaturated fatty acid were different according to the avian types, which may stimulus yolk efficiency throughout storage and protection against certain harmful effects for spermatozoa like cold shock, osmotic stress, sperm injuries, acrosomal defects and DNA integrity (**Hermansson and Axner, 2007**). Avian different types may provide different sources of egg yolks, so; the present study was designed to investigate the effect of four types of avian which were chicken, quail, turkey and duck EYs extenders for ram epididymal sperms individual motility, sperms live percentage and sperms abnormalities and storage in chilled temperature (4 Cº).

## MATERIIALS AND METHODS

### Animal samples and Study area:

The study was carried out in the laboratory of Artificial Insemination, College of Veterinary Medicine, University of Mosul, Mosul, Iraq (N: 36° 20' 24": E: 043° 07' 48") from September 2020 to November 2020. Sixteen pairs of testicles (n=32 testis/ semen samples ) from 16 mature Awassi rams aged between 1-4 years, slaughtered at Al-Sadoon abattoir were gained. The testes were transported in a cold box after washing with normal saline and antibiotics (penicillin-streptomycin) within 2 h after slaughtering.

### Epididymal Sperms collection:

The spermatozoa were obtained by cutting and squeezing the cauda epididymis at ambient temperature in a Petri dish. The semen was collected in a 15 ml glass graduated tube(**Abu *et al.*, 2016**). All epididymis were gently excised from the testis. Sperms were undergone for macroscopic and microscopic examination under the light microscope for Individual motility, live sperms and sperms abnormalities percentages were estimated using red eosin %5 and nigrosine %10 stains. These examinations of sperm parameters were evaluated before dilution for all tests samples (n=32) and after dilution with avian egg yolks at 0, 72, 144 hours.

### Sperms individual motility:

Sperm individual motility was estimated according to **Ferdinand *et al.*, (2012)**. Briefly, 5 µl of semen was assessed for motion under the microscope (40x) using coverslip and scored into 0-100 grades.

### Sperm live percentage and abnormalities:

A semen smear was prepared by mixing 2 µl semen and 10 µl Eosin-Nigrosin stain (10 gm of Nigrosin, 1.7 gm Eosin and 2.9 gm of sodium citrate in100 ml of distilled water). Sperm cells counted as alive that exclude strict exclusion of the stain (whitehead) and dead that stain eosin (redhead) against Nigrosin background (400x), calculating 200 sperms under the microscope after 2-3 minute slide dryness. The sperm's live percentage and abnormalities were estimated using the Eosin-Nigrosin staining as described by **Uysal and Buck, (2007).**

### Egg yolk extenders and semen dilution:

A total of 0.5 ml of spermatozoa volume was collected from the epididymis and diluted using sodium citrate 2.9% and completed to 1 ml, then divided into four aliquots (0.25 ml for each sample). Sperms were diluted in 1:10 (semen: extender) percentage ratio. Each type of egg yolk 10% which perpetrated ( by drawing 10 ml of yoks in sterile syringe) and adding to the extender, which consisted of egg yolk 10%, sodium citrate 2.9%, fructose 2.4gm, penicillin, and streptomycin (100.000 IU and 100 mg, respectively) in total 100 ml distal water (**Naoman and Taha, 2010**). The extender is added directly into the semen gradually to avoid a shock to the sperm. After 2-5 minutes of equilibration in the water bath, all extenders were examined for motility, live and dead sperm percentage, sperm abnormalities, and considered 0 hours, the samples in test tubes were moved in the refrigerator under 4 C and sperms examined after 72h, 144h, as affixed time under a light microscope.

### Statical analysis:

The results of the present study were expressed as mean + standard error. One-way ANOVA was used to compare data using Sigma Stat (Jandel scientific software V3.1). Duncan's Multiple Range Test was used to assess if there were any significant variations at P(<0.05).

## RESULTS

Data in Tables 1,2 and 3 shows the effect of different egg yolk types during different storage times on spermatozoa characteristics, including sperm individual motility, live sperms and sperm abnormalities percentage, at 0, 72 h, and 144 h of storage at 4ºC. In general, data show a well protective effect of quail EY on sperm characteristics followed by turkey EY, chicken and duck EYs, respectively after storage for 144h. Individual motility of the spermatozoa after 144 h of storage in quail EY extender was (36.00%± 1.20), which is higher than turkey EY (32.30%± 1.30).

Table 1: Percentage of Individual sperms motility of four avian egg yolks at 0, 72, and 144h of storage in 4°C.

| Quail EY | Turkey EY | Duck EY | Chicken EY | Time (hours) |
|---|---|---|---|---|
| 84.33 ± 1.70 [a] | 81.00 ± 2.40 [a] | 80.30 ± 1.70 [b] | 87.33 ± 1.50 [a] | 0 |
| 60.90± 2.70 [a] | 58.30± 2.90 [a] | 49.60± 1.80 [b] | 56.60± 2.60 [a] | 72 |
| 36.00± 1.20 [a] | 32.30± 1.30 [a] | 19.00± 1.50 [c] | 24.60± 2.30 [b] | 144 |

*Abc*: Different letters in same arrows indicate that the values are substantially different at (P<0.05).

However, these results were higher and significant (P< 0.05) when compared with chicken EY extender (24.60%± 2.30) and duck EY extender (19.00%± 1.50). Similar results of quail and turkey EYs were manifested in the percentage of live spermatozoa, which were (38.00%± 1.20), (33.30%± 1.30), (24.00%± 2.30), and (19.90%± 1.50), respectively. The duck EY was significantly lower (P< 0.05) when comparing the four egg yolks types after storage for 144 h. Sperms abnormalities percentages were higher in duck EY extenders when compared with the other three egg yolks types, but with no significant differences between them after storage at 0, 72, and 144 hours.

Table 2: Percentage of live sperms in four avian egg yolks at 0, 72, and 144h of storage in 4°C.

| Quail EY | Turkey EY | Duck EY | Chicken EY | Time (hours) |
|---|---|---|---|---|
| 86.30± 0.9 [a] | 85.66± 1.8 [a] | 81.60± 1.2 [a] | 88.33± 1.2 [a] | 0 h |
| 66.70± 2.4 [a] | 66.00± 2.9 [a] | 59.33± 1.8 [b] | 64.00± 2.1 [a] | 72 h |
| 36.00± 1.2 [a] | 32.30± 1.3 [a] | 19.00± 1.5 [b] | 24.00± 2.3 [b] | 144 h |

*Abc*: Different letters in same arrows indicate that the values are substantially different at (P<0.05).

Table 3: Percentage of sperms abnormalities in four avian egg yolks at 0, 72, and 144h of storage in 4°C.

| Quail EY | Turkey EY | Duck EY | Chicken EY | Time (hours) |
|---|---|---|---|---|
| 1.06± 0.6 [a] | 1.2± 0.1 [a] | 2.3± 0.1 [a] | 1.3± 0.0 [a] | 0 h |
| 7.1± 0.1 [a] | 7.4± 0.2 [a] | 9.1± 0.1 [a] | 7.9± 0.2 [a] | 72 h |
| 7.8± 0.1 [a] | 8.4± 0.2 [a] | 9.9± 0.2 [a] | 8.6± 0.2 [a] | 144 h |

*Abc*: Different letters in same arrows indicate that the values are substantially different at (P<0.05).

## DISCUSSION

In the present study, after 144 hours of storage, quail EY extenders a significantly higher percentage of individual motility (36.0%±1.2) and live sperms (38.0%±1.2)followed by turkey EY (32.3%±1.3 and 33.3%±1.3). However, individual motility values in chicken EY (24.6%±2.3) had significantly (P < 0.05) higher than the duck EY (19.0%±1.5), same result by (**Saieed *et al.*, 2018; Sunday *et al.*, 2018; Durand *et al.*, 2020**) who reported quail EY extender are best for ram semen storage when they recorded 66.67% and 40.85% motile sperms after 48 and 72 hours of storage which higher than duck EY (39.28%), turkey (38.00%) and chicken (36.85%), their result were attributed to composition of quail EY which has a lower ratio of polyunsaturated to saturated fatty acids compared to chicken EY (**Choi *et al.*, 2001**).

Saturated fatty acids are more stable than unsaturated fatty acids counterparts, this may be give quail EY the advantage of better protection capability which recorded in this study were are agreement results reported by **Trimeche *et al.*, 1997** who reported a higher percentage of motile and progressively undulating spermatozoa using quail EY compared with chicken EY extender in jackass; they suggested that the reason of high percentage of motile sperms with quail egg yolk extender because higher content of phosphatidylcholine, less phosphatidylethanolamine (**Sunday *et al.*, 2018; El-Shamary *et al.*, 2015**).

Our findings are in accordance with previous reports in buffalo (**Rawash *et al.*, 2020; Waheed *et al.*, 2012**), in buck (**Swelum *et al.*, 2018**), and bulls(**Achi *et al.*, 2017**), these authors reordered that sperm cells were better protected in media containing duck or chicken EY than media containing quail EY. Furthermore, the present result disagrees with (**Gholami *et al.*, 2010**) in rams and **Akhter *et al.*, (2010)** in bulls who registered that the epididymal sperm improve in pigeon EY. The comparable fertilization and embryonic growth values showed that the ram semen improved in quail, goose, turkey, ostrich, pigeon, duck and chucker EYs and give better sperms motility, viability, lower acrosomal abnormalities, and enhance membrane integrity when compared with chicken EY (**Ali *et al.*, 2013; Kulaksiz *et al.*, 2010**) which agreement with data of the present study.

Sperm abnormalities values after 72, 144h of storage time show no significant changes between all types of egg yolks, these data in line with (**Saieed *et al.*, 2018**) who registered 12.42%, 12.71%, 12.71% and 12.8% in quail, duck, turkey, and chicken EYs respectively with no differences between them.

There were discrepancies between the totals results between egg yolk types and animal types. The variations in sperm-membrane composition and egg yolk components of different avian species cause inconsistencies in the results of using egg yolks from different avian in semen extenders for different animals species-specific interactions may emerge (**Bansal and Bilaspuri, 2017**).

## CONCLUSION

The quail EY and turkey EY extender are better than chicken and duck EYs dilution of epididymal semen storage at 4C in Awassi rams.

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### Declaration of Conflicting Interests

The authors revealed that there was no potential conflicts of interest.

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