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Comparative study of semen physical characteristics and DNA sperm integrity in different breeds of falcon in Iraq

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Abstract--This study was conducted to evaluation of semen quality with the DNA integrity in the season of falcon in Iraq in the different breed (Saker falcon *Falco peregrinus*, and *Falco tinnunculus*) due to selection of breeding falcons used in the reproduction .the objective of study was to characterize semen quality in the deferent species of falcon. A total of 24 semen sample were collected from males by massage method. Semen parameters include mass activity, individual motility, dead and alive, abnormality and DNA integrity. Mass activity varied between breeds mass activity observed significant ($P\leq 0.05$) in the middle period of season, individual motility shows significant between initial, middle and later of season ($P\leq 0.05$). The evaluation of viability show no significant between breeds. Some teratospermia recorded, micro-head, tailless, and immature sperms in the different breeds with no significant differences. DNA integrity revealed significant among the individual males ($P\leq 0.05$). In conclusion the season of reproduction in Iraq play a role of semen viability and quality between different falcon breeds in Iraq.

Keywords--falcon, semen, sperm DNA integrity.

Introduction

In Iraq we have private natural preserve for breeding and proliferation of falcons, called Babylon Preserve for Breeding and Proliferation of Ostrich, Falcon and Deer. In this preserve, we bred three breeds of falcons, namely Gyr, Saker and Peregrine. Therefore, the present study was conducted to determine the effect of

falcon breed on certain semen quality traits of Gyr, Saker and Peregrine falcons (1)

Sperm characteristics vary between different peregrine falcon subspecies, individuals, and even between semen samples obtained from the same male due to the limited reproductive season and the difference in the methods used for semen collection (2) Early season semen is often of poor quality. Larger male falcons (e.g. Peregrine, Saker and Gyr) start to produce semen in their second season but reach peak production by their fourth season (3). Viability assessment is an important part of semen analysis, and various live/dead staining protocols have been used in semen of avian species. This differentiation is important for the prediction of fertility and the estimation of semen quality, especially in semen samples where the amount of motile spermatozoa is low (4). Therefore, this study concentrated about the effect of reproductive season with the evaluation of semen and hormonal assessment in three falcon .Sperm production varies seasonally; sperm concentration increases early during the breeding season, peaks in mid-season, and declines after. This pattern varies longitudinally. Numbers of spermatogonia, spermatids and abnormal spermatozoa are more likely to be present in the both early and late season ejaculations when testosterone levels are lower than normal (1).

Materials and methods

This study was conducted in the obstetrics lab of the veterinary college - University of Baghdad.

Animals

A total of 12 sexually matured males (3-4 years old) from three breeds of falcon (4 falcon males from each breed) , which were falcon Sakar , Falco peregrinus , Falco tinnunculus.(figure 1) The period of work begin in January to April that the season of falcon reproduction in Iraq .

Nutrition falcon

All males were fed on live pigeons which were introduced to males directly after been slaughtered and cleaned from feather especially the regions of chest and abdomen of pigeons as the falcon prefer to feed on live birds or fresh and recent slaughtered bird ,Or fed it on frozen birds after thawing to body temperature before slaughter.

Management of falcon

During the reproductive season (January – April) all males were trained on semen collection procedure by using special handling protocol for these birds which include extreme caution when dealing with these birds as they are raptor birds and have a very strong beak which can remove fresh meat very easily from the body and they have very strong claws used to grab prey and assist in cutting prey. After the male gets settled, repeated back massage is performed to ensure the male is calm on the one hand and on the other hand to start the stimulation

(Figure 2). The falcon is held in a professional way so that it is completely tied and prevents its movement so as not to harm the person who withdraws the semen.

Semen collection

Semen collection process begins through the simultaneous massage. Semen was collected using forced abdominal massage (5) of the higher part of the vent using the fingers of the left hand that caught the falcon's legs and behind the vent, using the right hand fingers. This process continues until the male papillae emergence from the vent, which indicates a good response from the male (Fig. 3). Using a pipette, the semen flowing out of the genital opening can be withdrawn. The semen collection procedure was completed, the semen samples were evaluated by using standard methods. Semen quality traits included in this study were mass and individual motility of sperm, dead and alive sperm, abnormal sperm DNA integrity of sperm.

Semen evaluation

Sperm motility

Mass activity

A drop of raw semen was put on slide (Fig. 4). and subjected to microscopical examination at 10x magnification, and scored into 1-5 scales (1+=no perceptible motion, 2+=few spermatozoa move without forming any waves, 3+=small slow moving waves, 4+=vigorous movement with moderately rapid wave and eddies and 5+=dense, rapidly moving waves and eddies) (6).

Individual motility

Determined by mixing one drop of raw semen with two drops of 2.9% sodium citrate solution, Semen was then diluted 1:100 (semen: extender) using modified Ringer's solution (sodium chloride: 68 g, potassium chloride: 17.33 g, calcium chloride: 6.42 g, magnesium sulphate: 2.5 g, sodium bicarbonate: 24.5 g, distilled water: 1000 ml), covered by cover slide, with subjected to microscopical examination at 40x magnification.

Dead and live sperm

Semen collected from falcon immediately tested in the slide one drop and stain sample by Eosin nigrosin stain and put cover slide, with subjected to microscopical examination (7), at 40X.

Sperm abnormalities

Abnormalities determined by collect one drop of falcon semen, stain sample by Eosin nigrosin stain and put cover slide with subjected to microscopical examination at 40X.

DNA integrity of sperm

Semen samples collected from falcons were stained by Aniline blue stain. One drop of collected semen mixed with one drop of aniline blue, covered by cover slide, followed by microscopical examination. Fragment DNA of sperm stained blue in color while the white color is normal sperm (Figure 8), (8).

Statistical Analysis

The Statistical Analysis System- SAS (2012) (9) program was used to detect the effect of difference factors in study parameters. Least significant difference -LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.



Figure 1: type three breed falcon



Figure 2: Stimulation of falcon by message



Figure 3: good response of male falcon





Figure 4: Simple of semen

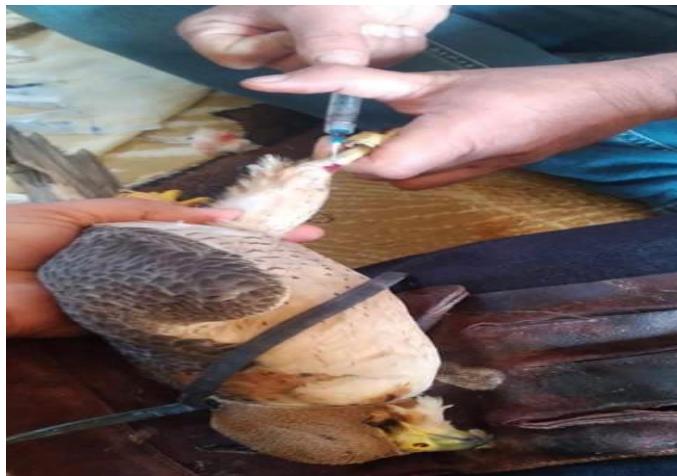


Figure 5: shows blood collection from metatarsal vein

Results and Discussion

This study was carried out the evaluation of fresh semen in three breeds of falcon with the testosterone hormone assay .The results shows Mass activity observed significant ($P \leq 0.05$) in the middle period of season (Table 1)

Table 1: Mass activity in three breed of falcons in breed season

Breed falcon	Mass activity in season stages			LSD value
	Initial	Middle	Later	
Saker falcon	2.75 \pm 0.16 A ab	4.25 \pm 0.28 AB a	2.5 \pm 0.12 A b	1.64 *
Falco peregrinus	3.25 \pm 0.22 A b	5.0 \pm 0.33 A a	3.25 \pm 0.17 A b	1.66 *
Falco Tinnunculus	2.25 \pm 0.15 A ab	3.5 \pm 0.18 B a	2.0 \pm 0.09 A b	1.41 *
LSD value	1.33 NS	1.47 *	1.37 NS	---

Means with different big letters in the same column and small letters in the same row are significantly different. * ($P \leq 0.05$).

While the individual motility shows significant between initial ,middle and later of season ($P \leq 0.05$) (table 2). Individual motility recorded in Gyr falcon (%) 68.8 ± 3.5 , in Saker falcon 74.2 ± 1.9 and in Peregrine falcon 83.9 ± 5.6 (1)

Table 2: The individual motility in three breed of falcons in breed season

Breed falcon	Individual motility in season stages			LSD value
	Initial	Middle	Later	
Saker falcon	60 ± 3.57 AB b	70 ± 3.31 B a	55 ± 2.46 B c	9.44 *
Falco peregrinus	65 ± 3.81 A b	80 ± 3.58 A a	65 ± 2.98 A b	8.61 *
Falco tinnunculus	52.5 ± 2.44 B b	70 ± 3.36 B a	55 ± 2.14 B b	8.07 *
LSD value	8.73 *	8.55 *	7.92 *	---

Means with different big letters in the same column and small letters in the same row are significantly different. * ($P \leq 0.05$).

The evaluation of the dead sperms in the three breeds gives no significant sperm stained by eosin nigrosin for dead and alive sperm figure 6 exam no significant between breeds and during the season . (10) used eosin blue stain(EB) and suggest EB as the most suitable stain for viability assessment in the semen of large falcons. In our study observed no significant between falcons through the season in dead and alive sperms .Table 3

Table 3: Dead and alive sperm in three breed of falcon

	Dead sperm			LSD value	Alive sperm			LSD value
	Initial	Middle	Later		Initial	Middle	Later	
saker falcon	25.0 ± 1.35	21.5 ± 1.08	24.5 ± 1.57	5.98 NS	75.0 ± 3.51	78.5 ± 3.04	75.5 ± 2.38	6.03 NS
Falco peregrinus	20.5 ± 0.78	21.0 ± 0.96	22.0 ± 1.35	4.59 NS	79.5 ± 3.67	79.0 ± 3.27	78.0 ± 3.74	5.14 NS
Falco tinnunculus	26.5 ± 1.57	21.0 ± 1.02	23.0 ± 1.09	5.63 NS	73.5 ± 2.96	79.0 ± 3.29	77.0 ± 3.19	5.04 NS
LSD value	6.85 NS	5.49 NS	5.71 NS	---	7.02 NS	6.41 NS	6.25 NS	---

NS: Non-Significant.

Abnormal sperms include immature sperm figure 7 with non significant in season and between breeds Table 4-A ,mico head figure 7 significant differences between breeds and season Table 4-B and tai less figure 7 the significant differences through seasonal periodin Falco peregrinus Table 4-C. A small percentage of sperm showed more than one abnormality simultaneously. Multiple defects included bent heads with multiple or coiled tails, macrocephalic headswith droplets, or multiple tails (11)

Table 4-A: Abnormalities of sperms // Immature in three breed of falcon

Breed falcon	Immature in stage season			LSD value
	Initial	Middle	Later	
Saker falcon	22 ±1.42	21 ±0.87	23 ±1.38	2.94 NS
<i>Falco peregrinus</i>	23 ±1.50	21 ±0.82	22 ±1.33	2.72 NS
<i>Falco tinnunculus</i>	22 ±1.43	19 ±0.63	22 ±1.33	3.08 NS
LSD value	3.04 NS	2.81 NS	2.55 NS	---
NS: Non-Significant.				

Table 4-B: Abnormalities of sperms // Micro head in three breed of falcon

Breed falcon	Micro head in stage season			LSD value
	Initial	Middle	Later	
Saker falcon	21 ±0.98 AB ab	18.5 ±0.59 B b	24 ±1.52 A a	5.22 *
<i>Falco peregrinus</i>	20 ±0.76 B a	18 ±0.64 B a	22 ±1.09 A a	4.37 NS
<i>Falco tinnunculus</i>	25 ±1.47 A ab	30 ±1.73 A a	20 ±0.79 A b	5.84 *
LSD value	4.95 *	6.73 *	4.52 NS	---
Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).				

Table 4-C: Abnormalities of sperms // Tailless in three breed of falcon

Breed falcon	Tailless in stage season			LSD value
	Initial	Middle	Later	
Saker falcon	25 ±1.46 A a	23.25 ±1.51 AB a	22 ±1.16 A a	3.04 NS
<i>Falco peregrinus</i>	23 ±1.15 A a	19 ±0.68 B a	23 ±2.09 A a	4.07 NS
<i>Falco tinnunculus</i>	22 ±1.33 A a	24 ±1.97 A a	22 ±1.26 A a	3.27 NS
LSD value	3.17 NS	4.88 *	3.61 NS	---
Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).				

Observed the variation between breeds in the season of reproduction in Iraq the season between January to April. The reproduction season between January to April seasonal bird whose reproductive cycle extends from February to May in Spain (12). Sperm integrity observed in table 5, the significant differences between Saker falcon and *Falco peregrinus* in later of reproductive period. the sperm DNA fragmented stained blue in color figure 8. Spermatogenesis in male birds of prey

depends on follicle-stimulating hormone (FSH), testosterone, the activity of Sertoli cells and their interaction with the spermatogonial stem cells. Seasonal testicular growth usually takes up to 45 days in the majority of raptor species, a period longer than ovarian growth in the female. FSH and LH, as well as testosterone, are essential for spermatogenesis (13- 14)

The DNA integrity of sperm is crucial for successful fertilization, development of the embryo, implantation, and pregnancy establishment (15). Sperm DNA integrity is thus regarded as a key indicator of spermatozoa reproductive potential (16, 17).

Table 5: Integrity of sperms in three breed of falcon

Breed falcon	DNS fragment %			LSD value	DNA normal			LSD value
	Initial	Middle	Later		Initial	Middle	Later	
Saker falcon	24 A a	19 A b	26 A a	4.85 *	76 A ab	81 A a	74 B B	5.91 *
Falco peregrinus	24 A a	20 A a	20 B a	4.03 NS	76 A a	80 A a	80 A a	4.67 NS
Falco tinnunculus	22 A a	20 A a	22 AB a	3.37 NS	78 A a	80 A a	78 AB a	3.28 NS
LSD value	2.27 NS	2.08 NS	4.92 *	---	2.27 NS	2.08 NS	4.92 *	---

Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).



Figure 6: dead and alive sperm .eosin nigrosin stain

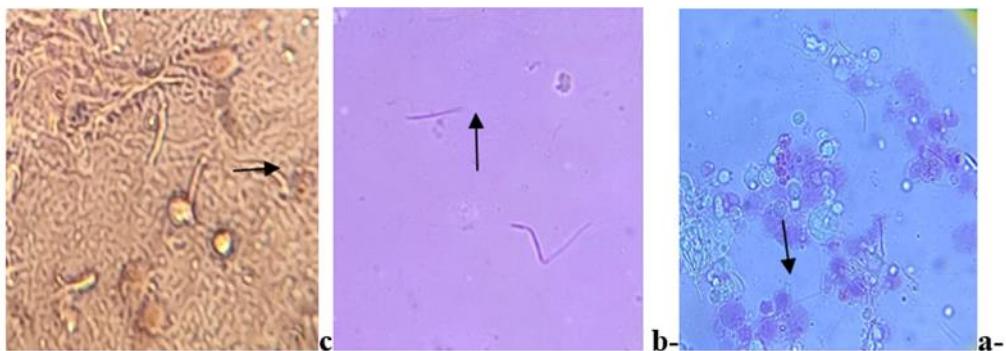


Figure 7:a-Shows immature sperm b- tailess sperm c- microhead

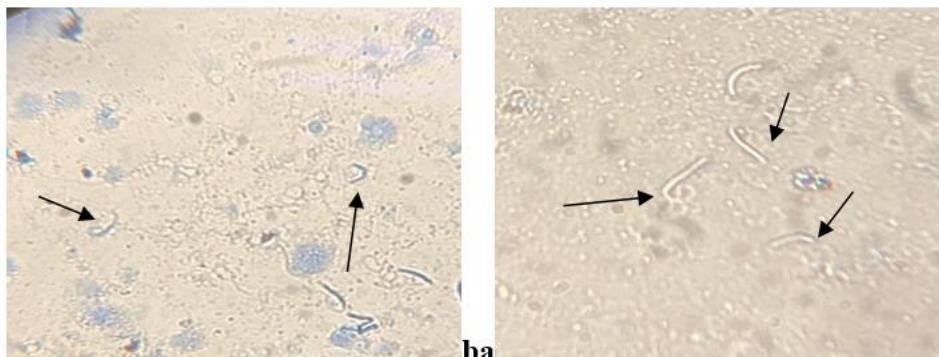


Figure 8. Shows a- normal sperms white in color . b- Fragmented DNA of sperm blue in color .Stained by aniline blue .40X

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