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Assessment of hormonal profile with the genetic variation in different breeds of falcon in Iraq

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***Abstract*---**This study was conducted to assessment the hormonal assay in the reproductive season of falcon in Iraq in the period duration between January to April, the animal used different breeds Saker falcon _ Falco peregrinus –and Falco tinnunculus), due to selection of breeding used in the reproduction .The objective of study was to characterize the level of testosterone hormone related with the androgen receptor gene polymorphism between deferent breeds of falcon. Blood samples were collected from metatarsal vein of males. Testosterone level recorded 9.15 ±0.74 ng, 9.23 ±0.58 ng, and 8.75 ±0.37 ng in the Falco peregrinus, gives at the result revealed significant differences between the breeds in the periods of season, initial ,middle ,and later subsequently. The molecular results revealed the polymorphism in the Falco tinnunculus. The genotype and allele frequency in the Falco peregrines and Flco Saker significantly at* (P≤0.05) in all genotypes the allele gives 4 (100%) to 0 (0%) in another one, while deferent read between genotypes in the Falco tinnunculus (P≤0.05), NS: Non-Significant, in the GG to TT, GG to AA, CC to GG, GG to AA, 2 (50%) and TT to GG at 1 (25%)**.** In conclusion, testosterone level observed in high level in the Falco peregrines compare with other breeds in this study. Androgen receptor gene sequences in the Falco tinnunculus peregrines more polymorphic than Falco Saker and Falco depended at the (National center for biotechnology information) NCBI.

***Keywords*---**Falcon, testosterone, androgen receptor gene, sequencing.

Introduction

During the reproductive season (January – April) all males were trained on semencollection procedure by using special protocol to handle these birds. Semen samples werecollected from all males on a fortnightly basis (1). The falcon displays some special reproductive features compared to other avian species. It is a monogamous and seasonal bird whose reproductive cycle extends from February to May (2). The beginning of its breeding season is mainly influenced by the photoperiod, according to the gradual increase in daylight hours (3). Sperm DNA differs from that in other cells of the body in terms of integrity and content, which is important for its fusion with the maternal genome (4). The DNA integrity of sperm is crucial for successful fertilization, development of the embryo, implantation, and pregnancy establishment (5).

Sperm DNA integrity is thus regarded as a key indicator of spermatozoa reproductive potential (6). The good condensation of the chromatin in the sperm during maturation of sperms is responsible to preserve the sperm DNA from damage during transport through the male to the female reproductive system (7). The classification of the sperm DNA abnormalities includes (i) epigenetic aberrations, (ii) mitochondrial DNA damage, (iii) Y-chromosome micro-deletions, (iv) DNA fragmentation, and (v) telomere attrition, depending on the location and form of the damage (8, 9). Increased abnormality of the sperm DNA has been documented in males with a decrease of semen parameters (10).

Materials and Methods

Blood collection: blood collection from metatarsal vein (Figure 1).

Figure 1: Blood collection from metatarsal vein

2- : Hormonal assay: Blood collection from metatarsal vein and measurement testosterone hormone in ABN-ALBATAR LABROTORY.

DNA Extraction

Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood gDNA Miniprep System (11). PCR for Androgen receptor gene 1: the program for AR gene 1 extracted was done by program table 1: Two primers for 1 exon used Table 2.

Table 1: PCR Program

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60, 65	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Table 2: primers for Androgen receptor gene

Primer	Sequence 5`-`3	AnnealingTemp.(°C)	Product size (bp
AR_1-F	GGATGGAGGAAAGCATAAGG	60	787
AR_1-R	GGTCTCACTAGGTCACTAAGA		

Agarose Gel Electrophoresis
After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification**.**

Standard Sequencing
PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequence, by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software.

Statistical Analysis
The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study (12-14)

Result and Discussion

Recent study was concentrated about the testosterone assessment in the season of Iraqi falcon between January and April. in the study (15) recorded the reproductive cycle extends from February to May in Spain 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, testosterone, was essential for spermatogenesis [16, 17]. Testosterone level recorded 9.15 ± 0.74 ng, 9.23 ± 0.58 ng, and 8.75 ± 0.37 ng in the Falco peregrinus, gives at (P≤0.05) significant deference's between the breeds in the periods of season, initial, middle, and later subsequently (Table 3**)**. 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)

The sequence of 12 samples that were obtained from falcons in iraq were compare against Falco rusticolus database sequences utilizing BLAST falcon androgen receptor gene AR-1 *gene* sequence .the amplified gene include the sequence of exon 1 (787bp) The results of current study reported SNP, A/C Exon 3 .215 , A/G Exon 3 .246 , A/C Intron 399 , A/T 437 , T/C 440, G/T 490 , G/A 525 , C/G , G/A595, G/T intron 6 n.26289, A/C 602 , C/Tintron 6 600 .N/T 628 and T/G 631 twelve falcons had genotype was observed in all falconsas shown , the data showed that falcons with all genotypes were significantly (P≤0.01) like AA compare with CC and so on with the other genotypes in table 4.

The genotype and allele frequency in the Falco tinnunculus and Flco Saker significantly at* (P≤0.05) in all genotypes the allele gives 4 (100%) to 0 (0%) in another one Table 5 and Table 7, while deferent read between genotypes in the Falco tinnunculus (P≤0.05), NS: Non-Significant, in the GG to TT, GG to AA, CC to GG, GG to AA, 2 (50%) and TT to GG at 1 (25%) Table 6.

The results revealed the polymorphism in the Falco tinnunculus compared with the two breeds Flco Saker and Falco peregrinus, the polymorphism between breeds seems to be the reason for testosterone hormonedifferent in the assay between three breed of falcon during season.

Table 3: Hormonal assay for three breed of falcon during season

Breed falcon	Testosterone level			LSD value
	Initial %	Middle%	later %	
Saker falcon	1.95 ±0.13ng B a	2.6 ±0.08ng B a	2.15 ±0.10ng B a	1.84 NS
Falco peregrinus	9.15 ±0.74ng A a	9.23 ±0.58ng A a	8.75 ±0.37ng A a	2.58 NS
Falco tinnunculus	3.18 ±0.18ng B a	3.56 ±0.26ng B a	3.15 ±0.17ng B a	1.42 NS
LSD value	1.66 *	1.72 *	1.67 *	---
Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).				

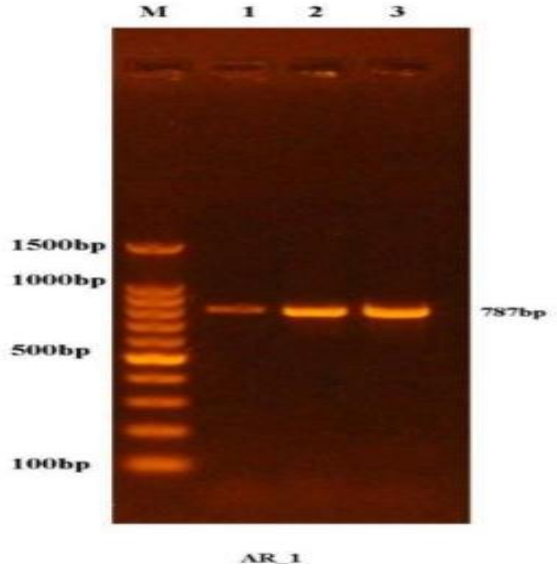

Figure 2: Results of the amplification of AR-1 *gene* of Falco samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-3 resemble PCR products

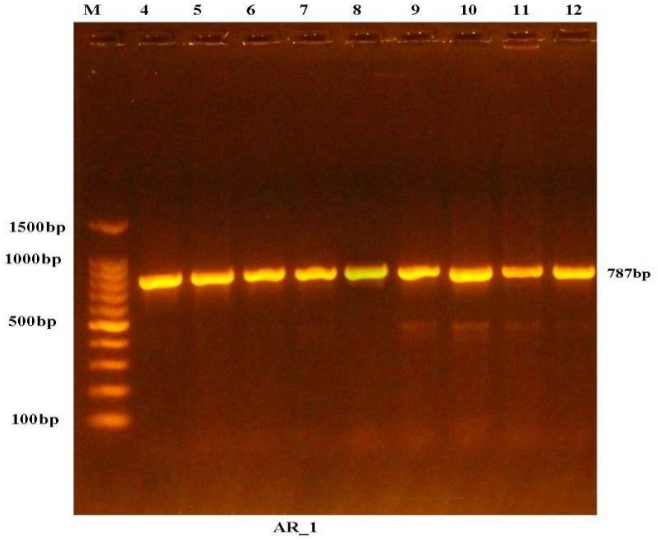

Figure 3: Results of the amplification of AR-1 *gene* of Falco samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 4-12 resemble PCR products.

Table 4: Genotype distribution and allele frequency of mutation

Locus	Genotype	Genotypic Frequency	Percentage (%)	Chi- Square	Allele frequency
A/C Exon 3	AA	9	75	8.335 **	A: 0.75
	CC	3	25		C: 0.25

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A/G Exon 3 246	AA	9	75	8.335 **	A: 0.75 G:0.25
	GG	3	25		
A/C Intron 399	AA	3	25	8.335 **	A: 0.25 C: 0.75
	CC	9	75		
A/T 437	AA	11	91	10.49 **	A: 0.91 T: 0.09
	TT	1	9		
T/C 440	TT	9	75	8.335 **	T: 0.75 C:0.25
	CC	3	25		
G/T 490	GG	10	83	9.962 **	G: 0.83 T: 0.17
	TT	2	17		
G/A 525	GG	10	83	9.962 **	G: 0.83 A: 0.16
	AA	2	16		
C/G	CC	10	83	9.962 **	C: 0.83 G: 0.17
	GG	2	17		
G/A 595	GG	10	83	9.962 **	G: 0.83 A: 0.17
	AA	2	17		
G/T intron 6 n.26289	GG	11	91	10.49 **	G: 0.91 T:0.09
	TT	1	9		
A/C 602	AA	10	83	9.962 **	A:0.83 C:0.17
	CC	2	17		
C/T intron 6 600	CC	6	50	1.00 NS	C:0.5 T:0.5
	TT	6	50		
T/N 628	TT	9	75	9.597 **	T:0.75 C: 0.17 G: 0.08
	CC	2	17		
	GG	1	8		
T/G 631	TT	4	33	9.485 **	T:0.33 G:0.67
	GG	8	67		
** (P≤0.01)					

Table 5: Genotype distribution and allele frequency of mutation in Saker falcon

Breed 1 Loci	Genotype	Genotypic Frequency	Chi- Square
A/C	AA	4 (100%)	3.998 *
	CC	0 (0%)	
A/G	AA	4 (100%)	3.998 *
	GG	0 (0%)	
A/C	AA	0(0%)	3.998 *
	CC	4 (100%)	
A/T	AA	4 (100%)	3.998 *
	TT	0 (0%)	
T/C	TT	4 (100%)	3.998 *
	CC	0 (0%)	
G/T	GG	4 (100%)	3.998 *
	TT	0 (0%)	
G/A	GG	4 (100%)	3.998 *

	AA	0 (0%)	
C/G	CC	4 (100%)	3.998 *
	GG	0 (0%)	
G/A	GG	4 (100%)	3.998 *
	AA	0 (0%)	
G/T	GG	4 (100%)	3.998 *
	TT	0 (0%)	
A/C	AA	4 (100%)	3.998 *
	CC	0 (0%)	
C/T	CC	4 (100%)	3.998 *
	TT	0 (0%)	
T/N	TT	4 (100%)	3.917 *
	CC	0 (0%)	
	GG	0(0%)	
T/G	TT	0(0%)	3.998 *
	GG	4(100%)	
* (P≤0.05).			

Table 6: Genotype distribution and allele frequency of mutation in Falco peregrinus

Breed 2 Loci	Genotype	Genotypic Frequency	Chi- Square
A/C	AA	1 (25%)	3.986 *
	CC	3 (75%)	
A/G	AA	3 (75%)	
	GG	1 (25%)	3.986 *
A/C	AA	3 (75%)	
	CC	1 (25%)	3.986 *
A/T	AA	3 (75%)	3.986 *
	TT	1 (25%)	
T/C	TT	1 (25%)	3.986 *
	CC	3 (75%)	
G/T	GG	2 (50%)	
	TT	2 (50%)	1.00 NS
G/A	GG	2 (50%)	1.00 NS
	AA	2 (50%)	
C/G	CC	2 (50%)	1.00 NS
	GG	2 (50%)	
G/A	GG	2 (50%)	1.00 NS
	AA	2 (50%)	
G/T	GG	3 (75%)	0.75 NS
	TT	1 (25%)	
C/T	CC	1 (25%)	3.986 *
	TT	3 (75%)	
T/N	TT	1 (25%)	
	CC	2 (75%)	0.556 NS

	GG	1 (25%)	
T/G	TT	1 (25%)	3.986 *
	GG	3 (75%)	
* (P≤0.05), NS: Non-Significant.			

Table 7: Genotype distribution and allele frequency of mutation in Falco tinnunculus

Breed 3 Loci	Genotype	Genotypic Frequency	Chi- Square
A/C	AA	4 (100%)	3.998 *
	CC	0 (0%)	
A/G	AA	4 (100%)	3.998 *
	GG	0 (0%)	
A/C	AA	0 (0%)	3.998 *
	CC	4 (100%)	
A/T	AA	4 (100%)	3.998 *
	TT	0 (0%)	
T/C	TT	4 (100%)	3.998 *
	CC	0 (0%)	
G/T	GG	4 (100%)	3.998 *
	TT	0 (0%)	
G/A	GG	4 (100%)	3.998 *
	AA	0 (0%)	
C/G	CC	4 (100%)	3.998 *
	GG	0 (0%)	
G/A	GG	4 (100%)	3.998 *
	AA	0 (0%)	
G/T	GG	4 (100%)	3.998 *
	TT	0 (0%)	
A/C	AA	4 (100%)	3.998 *
	CC	0 (0%)	
C/T	CC	1 (25%)	3.986 *
	TT	3 (75%)	
T/N	TT	4 (100%)	3.917 *
	CC	0 (0%)	
	GG	0 (0%)	
T/G	TT	3 (75%)	3.986 *
	GG	1 (25%)	
* (P≤0.05).			

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