Biometric Testicular and Hormonal Serum Profiles of Arabian Stallion during Breeding Season in Algeria

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Abstract

Several studies have shown relationship of biometric testicular, hormonal profiles and fertility. Objective of present research were to predict the fertility of stallion by biometric testicular and hormonal analysis. Measurement of the testis of adults Arabian stallions (n=36) from National Haras Chaouchoua of Tiaret, Algeria. The length (TL), the width (TW), the height (TH), and the scrotal width of the testicles (TSW) during three consecutive seasons (April: 2014-2016). From these measurements, the total testicular volume (TTV), the daily sperm output (DSO) and the daily sperm product (DSP) were calculated. The serum testosterone concentration, the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) were also evaluated by IMMUNOTECH® during April 2016.The scrotal width (TSW: 11.06 ± 1.55) cm, was very highly correlated to the age and the body weight of the stallions as well as with all the testicular parameters ($p<0.01$). The scrotal width (TSW) was correlated positively to testosterone, (r=0,14).The examination of the testicular size is an important criteria to estimate for selection the stallions with highest fertility potential.

Keywords: *Breeding season, Testicular measurement, Hormonal profiles, Stallion, Algeria.*

Introduction

In Algeria, infertility and sub-fertility are the major reasons for disposal of large number of breeding. The variable used to access fertility in stallion is the pregnancy rate, but when this measure is not available, others methods exist such as: the testicular measurements, and the hormonal analysis. A general physical examination should be included in the routine breeding soundness examination of the stallion; an assessment of general body condition is done first. **(Blanchard et al. 2011, Perumal et al. 2017, Rua et al. 2017, Houssou et al. 2018).**

The neuroendocrine system is responsible for the function and control of reproductive organs through signaling pathways that are facilitated and influenced by certain hormones. Reproductive variability among males of different species has been attributed to differences in circulating hormone concentrations: testosterone (TE), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (**McDonnell et al. 1995, MCkinnon et al. 2011, Roser 2000, Bollwein et al. 2008).**

Stallions are considered to be "long-day breeders" because their reproductive capacity is maximized during the seasons in which the photoperiod is increased. The testicular measurements of stallions increase during spring and summer, which leads to an increase in sperm production, libido and plasma hormone concentrations of intestinal-cell stimulating hormone (ICSH), follicle stimulating hormone (FSH), testosterone, inhibin and prolactin **(McKinnon et al., 2011).**

Clay and Clay (1992) and **Pickett et al. (1989)** reported that the reproductive activity of stallions is arranged by seasonality and photoperiods.

To our knowledge, there are few works on evaluation of the sexual function of the stallion in Algeria. For that purpose, our work will concern the measures of these criteria and their interpretation.

Materials and Methods

The study area

The study was realized at the National Haras of Tiaret, which is situated in approximately 300 km in the southwest of Algiers, created in 1877, on a surface of 800 hectars with a total of 250 horses, compound of two main races (Arabian and Barb).. The province of Tiaret is characterized by a continental climate with harsh winter, hot and dry summer, the rainfall of 300–400 mm per year on average.

Stallion samples

We worked during three season: April (2014-2016) on twelve Arabian stallions (n=36) in the national stud farm of Chaouchoua Tiaret (adults with an average age of 15,38±5.14, with body weight of (372,31 ±29,59) kg and an average BCS of (5,02± 0,21) according to the method of **(Henneke et al. 1983).**

Testicular measurements

After the clinical examination, the testis were measured with a caliper; exposed in figures (1-3). Total scrotal width (cm): (TSW); the mean testicular width (cm) of the both of the two testicles: (MTW); the left testicular width(cm): (LTW); the right testicular width (cm):(RTW);the left testicular length (cm): (LTL); the right testicular length(cm):(RTL);the left testicular height (cm): (LTH);the right testicular height (cm): (RTH).

Figure 1. Total scrotal width

Figure 2. Testicular length

Figure 3. Testicular height

Testicular parameters

Testicular weight (TW); albuginica weight (AW), the testicular parenchyma weight (TPW), Daily sperm output (DSO), Daily sperm product (DSP) and the total testicular volume (TTV) ; were calculated from the following formulas exposed in table 1. **(Rajak et al. 2013, Perumal et al. 2017, Rua et al. 2017).**

Table1. The testicular parameters estimates by testicular measurement.

Testicular parameters	Formulas
The testicular weight (gramme)	TW = 71 (Total scrotal width)-140
The albuginica weight	AT =0,145 x (weight calculated) +2,52
the testicular parenchyma weight	TPW= TW-AT
DSO(Total scrotal width in mm)	$(0,066xTSW-3,36).10^9$
DSP (Total scrotal width (in mm)	$(0,093xTSW-4,88).10^9$
The testicular volume right or left (ml)	TV= 0.5233 x Lenght/2 x width/2 x hight/2
The total te ular volume	TTV = TVL+TVR.

Hormonal analysis

Serum hormonal concentration were analyzed from blood samples from each stallion one a month, collected in the morning (11h30) using tubes (Vacutainer®) The samples were collected and transported to the laboratory testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations were determined through use of a solid-phase radioimmunoassay (RIA) using acommercial diagnostic kit from Immunotech® (Automat Perkin Elmer).

Statistical analysis

Data were analyzed using SPSS 20 and expressed as the mean ± standard error of mean (SD) min; max and variance. Data collected were subjected to various statistical tools in a one-way analysis of variance followed by the Student Newman–Keuls multiple comparison test, was used to study the effect of age and the total scrotal width on testicular parameters and hormonal levels.

Y= = b + b X + b X (quadratic model)

Results and Discussion

The results of different testicular parameters were presented in tables N° 2, 3, 4, 5, 6 and figure 4. To our knowledge, there is little or no works on the estimation of the stallion fertility by testicular measurements or hormones analysis in Algeria.

The scrotal width of the testicle (TSW): 11.06 ± 1.55cm. The testicular biometry were evaluated (left and right separately) and there were a differences between left and right ($p < 0.001$), (Table 2).

The testicular biometry there were differences between left and right ($p < 0.001$) in large horses breed there has been reported a tendency for the left testicle to be large than the right testicle (**Morel 1999, Mambrini et al. 2010; Rua et al. 2017 ; Hafez and Hafez 2004)** also observed the same, suggesting that this occurs due to the earlier development of the left testicle in relation to the right testicle. (Table 3).

Tibary et al. (2005) and **Blanchard et al. (2011)** reported the difficulty of approached the horse of the right-hand side.

Table 2. Morphometric testicular in Arabian stallion during (2014-2016).

Variable	Mean	Min	Max	SD	Variance	Std. Error
Age	15.38	5.00	25.00	5.14	26.41	0.74
Body weight (BW),	372.31	328.00	426.00	29.59	875.67	4.27
BCS	5.02	4.00	6.00	0.21	0.25	0.04
RTW (cm)	5.52	3.00	6.10	1.11	1.23	0.16
LTW (cm)	6.08	4.00	6.50	0.96	0.93	0.14
MTW (cm)	5.85	4.00	6.44	1.09	1.19	0.16
TSW (cm)	11.06	8.00	12.00	1.55	2.40	0.22
RTL (cm)	7.50	5.00	10.00	1.38	1.92	0.20
RTLL. t. g (cm)	7.21	4.00	9.00	1.60	2.55	0.23
RTH(cm)	5.85	4.00	8.00	0.77	0.60	0.11
LTH (cm)	5.98	4.00	7.00	0.81	0.66	0.12
TW gr	501.40	456.00	538.00	74.70	10511.41	15.62
ATgr	85.73	69.00	124.00	15.68	245.73	2.26
TPW gr	465.67	388.00	485.00	82.53	8561.68	13.36
DSO 10^9	3.70	2.00	5.00	1.02	1.12	0.15
DSP 10^9	5.40	3.00	6.50	1.54	2.37	0.22
TTVml	257.54	98.00	429.00	86.29	9272.13	13.90

*Total scrotal width (cm): (TSW); the mean testicular width (cm): (MTW); the left testicular width (cm): (LTW); the right testicular width (cm): (RTW); the left testicular length (cm): (LTL); the right testicular length (cm): (RTL); the left testicular height (cm): (LTH); the right testicular height (cm): (RTH); daily sperm output:DSO(10^9; daily sperm product: DSP (10^9testicular parenchyma weight(TPW) the testicular weigh(gr): (TW); albuginica weight: (AT) the volume testicular total (ml):(TTV), * Significant at P <0.05** Significant at P <0.01*

Table 3. The variance between the right testicle width (RTW) and the left testicle (LTW)

	t	ddl	p
RTW (cm)	33.395	47	0.000
LTW (cm)	40.698	47	0.000

In the present study, the various measurements of testis size were highly correlated with each other, especially with (TSW). All correlation coefficients were highly significant ($p<0.01$). These results were in agreement with those reported by **Tibary et al. (2005); Samper et al. (2007) ; Hafez and Garner (2000) ; Rua et al. (2017).**

The correlation (Table 4) Reveals that the widths of both right and left testicles are very correlated between their ($r=0.85$, $p<0.01$). The testicular volume presents (TTV) an average value of 257.54 ± 86.29 ml. The weight of both testicles is of (TW) 501.40 ± 74.70 gr.

Correlations coefficients (Table 4) showed that various measurements of the testis correlated with each other ($P < 0.01$). The scrotal width (TSW) correlated significantly with all testicular measurements and age of stallions ($P < 0.01$). The daily sperm output (DSO): (3.70± 1.02) 10^6 and the daily sperm product (DSP) (5.40 ± 1.54) 10^9 predicted from the scrotal width testicular follow consequently the same evolution in function of age (Table 2 and 4).

According to **Pickett (1993)** and **Tibary et al. (2005),** the number of the sperm cells which a stallion can produce is a indicating parameter to estimate its fertility, it depends on the quantity of the functional testicular, and determines the number of mares minting by a stallion.

Figure 4 expresses the relationship between the mean testicular width (MTW) and age. The estimated regression equation showed high coefficient of determination R2 = 60%.

According **Elwish and Jones** cited by **(Clément et al. 1998)** the testicular size increases until 5 years, more slowly until 12 - 15 years and declines then. They found a regression equation which connects the mean testicular width (Y) according to the age (X) in years is the following one:

$Y (cm) = 4.498+0.143 X-0.006 X^2$

Regression equation of Arabian stallion in our experience is:

$MTW = 5.586 + 0.193 Age - 0.10 Age^2$

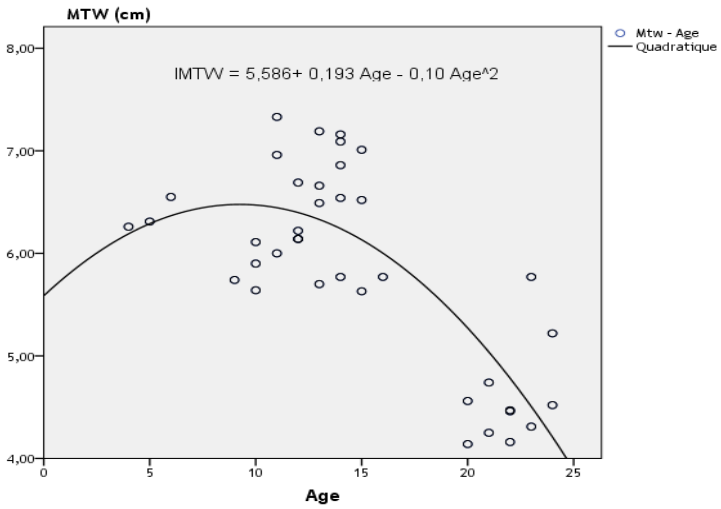

Figure 4. The relationship between the mean testicular width (MTW) and age.

Table 4. Coefficient of correlation among morphometric testicular in Arabian stallion during (2014-2016)

r	Age	LTW (cm)	RTW (cm)	MTW	TSW (cm)	TW gr	AT gr	TPW gr	DSO 10^9	DSP 10^9	VTT ml
Age	1	-0.52**	-0.52**	-0.57**	-0.58**	-0.58**	-0.58**	-0.58**	-0.62**	-0.60**	-0.66**
Body weight (kg)	-0.55**	0.53**	0.38**	0.53**	0.44**	0.43**	0.44**	0.43**	0.44**	0.42**	0.54**
BCS	-0.45**	0.42**	0.29*	0.43**	0.42**	0.44**	0.44**	0.44**	0.49**	0.44**	0.45**
MTW (cm)	-0.57**	0.87**	0.84**	1	0.81**	0.84**	0.84**	0.84**	0.76**	0.83**	0.81**
TSW (cm)	-0.58**	0.82**	0.72**	0.81**	1	0.97**	0.97**	0.97**	0.94**	0.95**	0.79**
RTW (cm)	-0.52**	0.85**	1	0.84**	0.72**	0.74**	0.74**	0.74**	0.65**	0.73**	0.79**
RTL (cm)	-0.57**	0.72**	0.68**	0.66**	0.65**	0.67**	0.67**	0.67**	0.57**	0.65**	0.85**
LTL (cm)	-0.55**	0.69**	0.61**	0.66**	0.75**	0.71**	0.71**	0.71**	0.65**	0.71**	0.88**
RTH (cm)	-0.28*	0.11	0.01	0.07	0.04	0.13	0.12	0.13	0.08	0.10	0.41**
LTH (cm)	-0.54**	0.43**	0.41**	0.47**	0.44**	0.47**	0.47**	0.47**	0.41**	0.48**	0.70**
TW gr	-0.58**	0.82**	0.74**	0.84**	0.97**	1	1.00**	1.00**	0.93**	0.97**	0.81**
TPW gr	-0.58**	0.82**	0.74**	0.84**	0.97**	1.00**	1.00**	1	0.93**	0.97**	0.81**
DSO 10^9	-0.62**	0.73**	0.65**	0.76**	0.94**	0.93**	0.93**	0.93**	1	0.94**	0.72**
DSP 10^9	-0.60**	0.79**	0.73**	083**	0.95**	0.97**	0.97**	0.97**	0.94**	1	0.80**
TTV ml	-0.66**	0.84**	0.79**	0.81**	0.79**	0.81**	0.81**	0.81**	0.72**	0.80**	1

*Total scrotal width (cm): (TSW); the mean testicular width (cm): (MTW); the left testicular width (cm): (LTW); the right testicular width (cm): (RTW); the left testicular length (cm): (LTL); the right testicular length (cm): (RTL); the left testicular height (cm): (LTH); the right testicular height (cm): (RTH); daily sperm output:DSO(10^9; daily sperm product: DSP (10^9testicular parenchyma weight(TPW) the testicular weigh(gr): (TW).); the volume testicular total (ml):(TTV), * Significant at P <0.05** Significant at P <0.01*

Serum testosterone concentration in this study are comparable to those of the **Cox et al. 1973, Amann 1993, Burns et al. 1982, Botton et al. 2008 and Bollwein et al. 2008.**

Serum testosterone concentration showed low and moderate correlations with testicular characteristics**. McKinnon et al. (2011)** related that there is correlation between testicular volume (testicular parenchyma) and sperm output and between testicular volume and testosterone.

Roser 2008 reported that the FSH influence the production of estrogens of the cells of Sertoli. Which explains the correlation between the testosterone and FSH (r= 0.24 p>0.05).

Negative correlation between (TE) and LH (-0.40; p>0.05), An elevated testosterone level in the bloodstream feeds back on the hypothalamus and anterior pituitary gland to suppress discharge of GnRH and thus LH, causing Leydig cells to produce less testosterone and FSH concentrations must be adequate to stimulate a Sertoli cell environment conducive to spermatogenic cell support and development. (**Blanchard and Varner 1996**). According to **Amann (1993)** the secretion of the LH is pulsatile, with irregular episodes of secretion produced every 2-4 hours, where from the difficulty measuring the basal rate of the LH.

The overall results of hormonal analysis of twelve Arabian stallion during April 2016 were presented in the table 5 and 6.The testosterone (TE) concentration 0.38± 0.26 ng/ml. follicle-stimulating hormone concentration (FSH) : 0.28 ±0.09 UI/L and

luteinizing hormone concentration (LH) : 0.03 ± 0.01 UI/L. Correlations coefficients (Table 6) showed that The scrotal width (TSW) correlated significantly with de LH (r= -0.77, p<0.01) and positively with (TE) and (FSH) (r= 0.14. 0.50, p >0.05).

Table 5. Results of hormonal concentration of 12 Arabian stallion (April 2016)

	Mean	Min	Max	SD	Variance	Std.Error
TE ng/ml	0.38	0.16	1.06	0.26	0.07	0.08
FSH UI/L	0.28	0.22	0.51	0.09	0.01	0.03
LH UI/L	0.03	0.01	0.04	0.01	0.00	0.00

TE : testosterone; follicle-stimulating hormone: (FSH) and luteinizing hormone: (LH)

Table 6. Coefficient of correlation among hormonal concentration in Arabian stallion during 2016

r	Age	TSW (cm)	PT gr	PPT gr	DSO 10^9	DSP 10^9	TTV ml	TE ng/ml	FSH UI/L	LH UI/L
TE ng/ml	-0.59	0.14	0.02	0.02	-0.03	-0.09	0.34	1	0.24	-0.40
FSH UI/L	-0.37	0.50	0.59	0.59	0.59	0.59	0.58	0.24	1	-0.46
LH UI/L	0.40	-0.77**	-0.69*	-069*	-.061	-0.55	-0.75*	-0.40	-0.46	1

*Total scrotal width (cm): (TSW); daily sperm output: DSO (10^9); daily sperm product: DSP (10^9); the volume testicular total (ml), VTT the testicular weigh (gr): (TW).,TE : testoterone;,follicle-stimulating hormone: (FSH) ; luteinizing hormone: (LH) * Significant at P <0.05** Significant at P <0.01.*

Conclusion

It may be concluded that the examination of the testicular size is an important criteria for selection the stallions with highest fertility potential. The study managed on twelve Arabian stallions during three consecutive season (April 2014-2016) allowed us to assert the strong correlation between the scrotal width with the various testicular parameters and hormonal levels; to place the period the most suited to the reproduction under our latitudes during April. However, an analysis of all the studied criteria, over the year, will allow us to appreciate better the level of seasonality of races under our latitudes and to plan a possible national program of artificial insemination.

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