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Variance in Stallion Testicular Parameters Between the Arabian and Barb breeds

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Abstract

The present study was carried out to characterise and quantify the level of fertility by analysis the effect of breed on testicular parameters stallions (Arabian versus Barb). Testicles were measured with a calliper total scrotal width (TSW), the left and the right testicular width (LTW, RTW), the left and the right testicular length (LTL, RTL) and the left and the right testicular height (LTH, RTH) respectively. The experiment was conducted during a period of a year on twelve Arabian stallions (n=144) and six Barbian stallions (n=72) clinically healthy raised in National Haras Chaouchoua of Tiaret, Algeria. From these measurements, the total testicular volume (TTV), testicular parenchyma weight (TPW) the testicular weigh (TW), the daily sperm output (DSO) and the daily sperm product (DSP) were calculated. Results obtained were tabulated and statistical analysis was performed using the statistical package SPSS 20.0 (SPSS Inc., Chicago, IL). The breed and the season effect was performed by using One-way ANOVA analysis. The level of significance was set at $P < 0.05$. There was highly significant difference ($p < 0.01$) in the DSP between breeds (4.12 ± 1.16 versus 4.56 ± 1.12) respectively in Arabian and Barb stallions. There was a significant influence of breed ($p < 0.05$) on some testicular parameters such (TTV, TW, TSW, TPW and DSO). Our study allowed us to better understand the distinction between Arabian and barbed stallions by the size of their testicles and their sperm production. The Barb stallions have higher testicular parameters compared to the Arabian stallions caused by genetic effect.

Keywords: Breed, equine, fertility, reproduction, season.

Introduction

Horses with good phenotypic and genetic characteristics are purchased for considerable amounts of money worldwide (Houssou et al., 2021). Infertility and sub-fertility are the major reasons for the disposal of a large number of breeding; methods exist to access the fertility in stallion such as the testicular measurements and the hormonal analysis (Houssou et al., 2021). It is suggested to include a general physical examination in the stallion's routine examination; an assessment of general body condition is carried out first, and ultrasound or testicular caliber measurements are also commonly requested (Rua et al., 2017; Houssou et al., 2018). Stallions are considered "long-day breeders" because their reproductive capacity is greatest during seasons of increased photoperiod. Stallion testicle size increases in spring and summer Houssou et al. (2021) reported that stallion reproductive activity is sequenced by season and photoperiod. Historically, the breeding season in the northern hemisphere extends from February 15 to July 15 (Allen and Wilsher, 2011).

The purpose of a fertility examination is to estimate a stallion's capability as a sire. Ideally, the testing process should produce sufficient information about a stallion to permit approximation of the number of mares to which that stallion can safely be booked during a breeding season. An indispensable ingredient in this formulation is the stallion's estimated daily sperm output (DSO). Numerous other factors also must be considered before made a reasonable judgment about "stallion book" (Houssou et al., 2021).

There is a lack of information about the characteristics associated with reproductive efficiency as the testis biometry and sexual behaviour of the Algerian stallions; therefore, it needs for more research, especially for the selection of the stallions with the highest fertility potential. The differences in fertility could potentially be explained by the genetic variability and its correlation with the semen characteristics (Alamaary et al., 2019; Houssou et al., 2020). There are few works on the evaluation of the testicular function of the stallion in Algeria (Houssou et al., 2021). Our work will focus on analyzing the measurements of testicular parameters and interpreting them in order to understand and to monitor the daily sperm output according the breed.

Materials and Methods

The Study Area

The research was carried out at the National Haras of Tiaret, located around 300 kilometers southwest of Algiers. Established in 1877, the Haras covers an area of 800 hectares and more than 250 horses, consisting of two primary breeds (Arabian and Barb). Tiaret province is characterized by a continental climate with harsh winter, hot and dry summer, the rainfall of 300–400 mm per year on average. Over the course of one year (2016-2017), we conducted research during both the breeding season (February-June) and non-breeding season (July-January) on a total of 144 Arabian stallions and 72 Barb stallions at the national stud farm Chaouchoua of Tiaret. These adult stallions had a Body Condition Score (BCS) of 5.02 ± 0.21 based on the method developed by Henneke et al. (1983). Body weight measurements were carried out in accordance with the Algerian horse population study (Houssou et al., 2020).

Testicular parameters

After the clinical examination, the testicles were measured with a calliper. Total scrotal width (cm): (TSW); the left testicular width(cm): (LTW); the right testicular width (cm):(RTW); the mean testicular width (cm) of the both of the two testicles: (MTW); the

left testicular length (cm): (LTL); the right testicular length(cm):(RTL); the left testicular height (cm): (LTH); the right testicular height (cm): (RTH). Testicular weight (TW); albuginea weight (AW)(figure 1), 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, according to the method of **Tibary et al. (2005)**, **Rua et al. (2017)** and **Houssou et al. (2021)**.

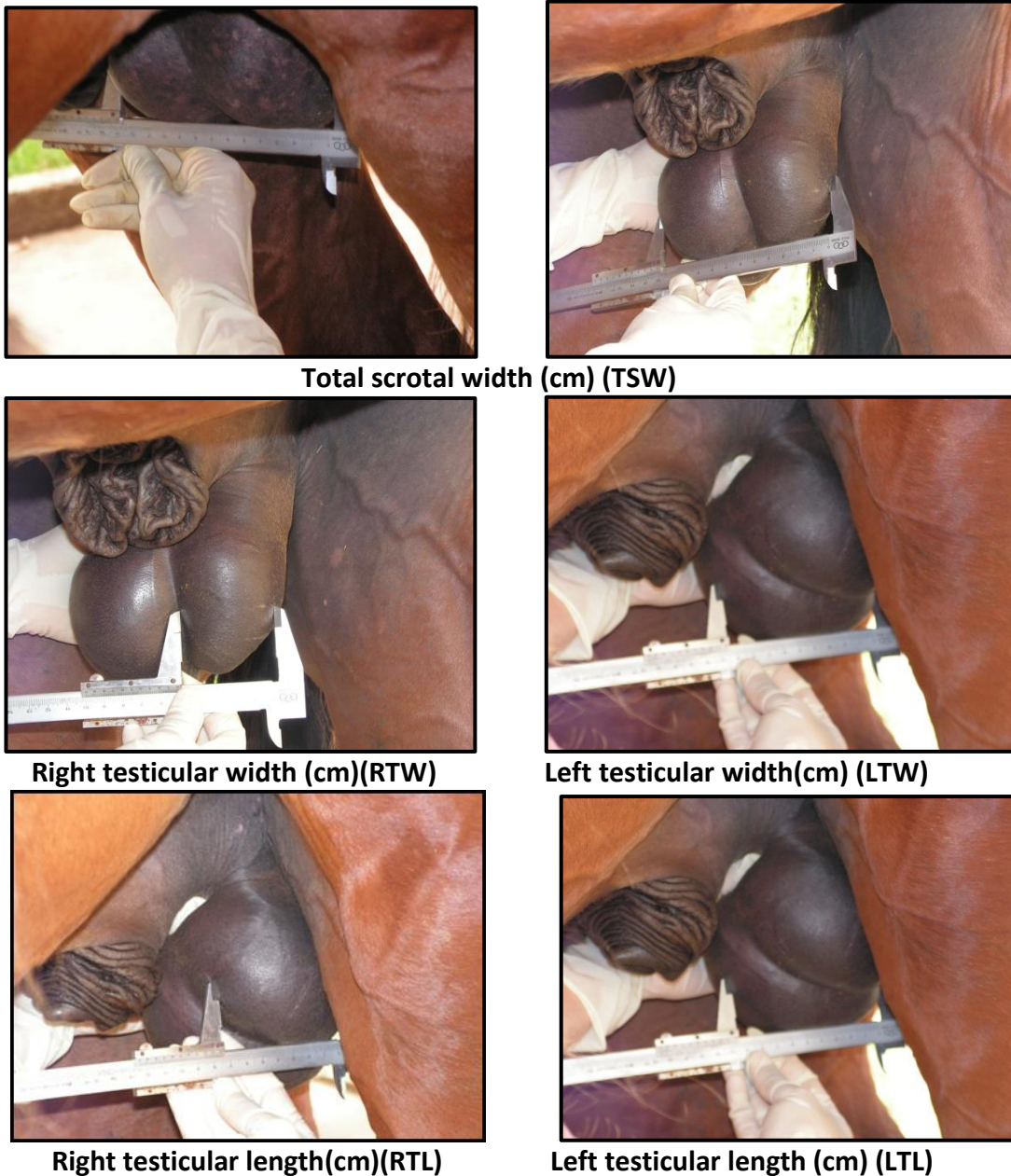


Figure 1. Testicular parameters

Statistical analysis

Data were analyzed using SPSS 20 and expressed as mean \pm standard error. The collected data were subjected to various statistical tools such as one-way analysis of variance, and then the Student-Newman-Keuls multiple comparison test was used to test the effects of breed on testicular parameters in order to study the effect of breed on testicular parameters. Differences with values of ($p < 0.05$ and $p < 0.01$) were considered to be statistically significant.

Ethical statement

The animals were studied in accordance with the ethical principles of animal experimentation and international animal welfare guidelines (Terrestrial Animal Health Code 2019, section 7. Art 7.12.1) and national executive decree No. 95-363 of November 11, 1995 (Algeria).

Results and Discussion

Testicular parameters: Data

Regarding testicular biometry are shown in table 2. The analysis of the variance indicates that the breed factor had a significant influence on the body weight except (BCS) and on the testicular parameters ($p < 0.05$; $P < 0.01$) except for RTW and RTL (Table 1). Therefore, this study allowed us to insist on the interest of identifying the breed of stallions for assessing the number of mares that will be mated by stallions.

Table 1. The effect of the breed testicular parameters in stallions.

Variables	Breed		p
	Arabian	Barb	
N	144	72	
BW (kg)	385.11±30.12	401.48±35.48	0.041
BCS	5.00±0.51	5.20±0.91	0.757
RTW (cm)	4.97±0.29	4.99±0.78	0.852
LTW (cm)	5.05±0.74	5.27±0.78	0.035
MTW (cm)	5.01±0.42	5.17±0.55	0.021
TSW (cm)	10.06±0.58	10.56±1.17	0.112
RTL (cm)	6.88±0.61	6.91±0.94	0.358
LTL (cm)	6.85±1.02	7.07±0.83	0.024
RTH (cm)	5.54±0.81	5.73±0.51	0.007
LTH (cm)	5.45±0.84	5.81±0.43	0.025
TW (gr)	503.55±52.74	547.48±67.91	0.005
AT (gr)	85.61±12.00	91.71±12.17	0.040
TPW (gr)	418.94±70.74	456.76±52.72	0.005
DSO 10⁹	3.53±0.77	3.89±0.78	0.003
DSP 10⁹	5.04±1.09	5.40±1.08	0.002
TTV (ml)	216.61±75.39	248.40±83.80	0.004
TW / BW	0.013±0.0001	0.012±0.0001	0.012

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 (10^9) testicular parenchyma weight (TPW) the testicular weigh(gr): (TW); albuginica weight: (AT) the volume testicular total (ml):(TTV).

The month affect significantly ($p<0.001$) testicular parameters (Table 2). We found that April and May were the best months for stallions breeding with higher DSP, DSO, and other testicular measurements. **Turner (2019)** reported that in breeds allowing artificial insemination, semen cryopreservation is recommended for a genetically valuable stallion when the animal is at the peak of its fertility.

Table 2. The effect of the month on body and testicular parameters in stallions

Month	MTW(cm)	TSW (cm)	TW (gr)	DSP 10^9	DSO 10^9
P	<0.001	<0.001	<0.001	<0.001	<0.001
February	5.14±0.56	9.47±0.84	532.36±59.64	4.19±0.83	3.89±0.77
March	5.20±0.44	9.78±1.01	554.05±70.81	4.39±1.21	4.89±0.78
April	6.55±1.12	11.17±1.4	603.41±99.15	5.01±0.86	4.01±0.52
May	6.01±0.68	11.19±1.31	654.75±93.18	5.04±0.22	4.29±0.72
June	5.06±0.53	9.86±0.93	560.03±66.18	4.43±0.55	3.89±0.78
July	5.06±0.37	10±0.89	569.99±63.52	4.11±0.61	3.88±0.78
August	4.9±0.48	9.57±0.7	539.2±49.71	4.04±0.15	3.21±0.22
September	5.08±0.44	9.6±0.99	541.55±69.99	4.02±0.92	3.04±0.28
October	5.06±0.43	9.44±0.93	530.39±66.3	3.99±0.84	2.89±0.78
November	4.84±0.46	9.23±1	515.49±71.13	3.95±0.66	2.59±0.78
December	4.84±0.41	9.17±0.84	511.03±59.39	3.94±0.98	2.55±0.71
January	5.11±0.44	9.86±0.97	560.35±69.07	4.02±0.75	2.85±0.92

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 (10^9)testicular parenchyma weight(TPW) the testicular weigh(gr): (TW); albuginica weight: (AT) the volume testicular total (ml):(TTV)

In the present study, the various measurements of testis size are highly correlated with each other, especially with (TSW). All correlation coefficients were highly significant ($p<0.01$) (Table 3).

Table 3. Correlation Coefficient among morphometric testicular in stallions

r	Age	MTW	TSW (cm)	TW gr	AT gr	TPW gr	DSO 10 ⁹	DSP 10 ⁹	VTT ml
Age	1	-	-	-	-	-	-	-	-
MTW (cm)	0.57**	1	0.81**	0.84**	0.84**	0.84**	0.76**	0.83**	0.81**
TSW (cm)	0.58**	0.81**	1	0.97**	0.97**	0.97**	0.94**	0.95**	0.79**
TW gr	0.58**	0.84**	0.97**	1	1.00**	1.00**	0.93**	0.97**	0.81**
TPW gr	0.58**	0.84**	0.97**	1.00**	1.00**	1	0.93**	0.97**	0.81**
DSO 10 ⁹	0.62**	0.76**	0.94**	0.93**	0.93**	0.93**	1	0.94**	0.72**
DSP 10 ⁹	0.60**	0.83**	0.95**	0.97**	0.97**	0.97**	0.94**	1	0.80**
VTT ml	0.66**	0.81**	0.79**	0.81**	0.81**	0.81**	0.72**	0.80**	1

Body weight (Kg): (BW); 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(10⁹); daily sperm product: DSP (10⁹) testicular parenchyma weight (TPW); the testicular weigh(gr): (TW); the volume testicular total (ml):(TTV), * Significant at P <0.05. ** Significant at P <0.01

Among the parameters evaluated, there was no significant difference of the breed on several testicular measurements in agreement with various authors described in the literature by **Tibary et al. (2005)**. Likewise, **Santos et al. (2022)** reported that testicular biometric pattern and size is influenced by genetic inheritance, body score, feeding, age, among other factors.

Tibary et al. (2005) reported that the stallion breeds a standard number of mares 30 to 45 mares by natural service or 120 to 140 mares by artificial breeding during a typical

breeding season of 135 to 150 days). Barb stallions with higher DSP and DSO can cover more mares than Arabian stallions in one year.

A decrease in testicular volume, sometimes associated with changes in the palpable texture of the testicles, is a hallmark of the disease. Ideally, routine testicular measurements should be obtained on all breeding stallions beginning when the stallion is in its prime to establish a normal baseline testicular volume. Because volume is a cubic measurement, even small errors in each linear measurement will have a significant effect on the calculated volume (**Turner, 2019**).

The researchers revealed found that, the daily nutritional requirements of stallions were 25% greater than the non breeding-season nutritional requirements of adult stallions. Although research on stallion diet is limited, it is clear that stallion registration, age, behaviour, body condition, general health, regular handling of the stallion and the level of free or forced exercise can all influence a stallion's performance. should be fed (**NRC, 1989**). In fact, stallions may experience weight loss and decreased appetite during this period (**NRC, 1989**). Indeed, during this period the stallions may be subjected to a loss of weight and decreased appetite (**Tibary et al., 2005; Hurtgen, 2009**). According to **Gentry (2004)**, to ensure adequate reproductive activity in mammals, a minimum level of physical condition is required. **Henneke et al. (1983)** Body condition also affects animal productivity, health, and reproduction (**Gentry et al., 2004**). Obesity in horses is defined as a body condition score (BCS) >7 (**Henneke et al., 1983**). In our study, stallions had an intermediate BCS (Table 1). Research shows that nutrition plays an important role in the reproductive performance of different species. Obesity has a significant impact on stallion performance; sperm reserves and quality are reduced; do not forget that it predisposes stallions to laminitis (**Hurtgen, 2009; Tibary et al., 2005**).

There was a difference between the left and the right testicle size. In horse breeds, there has been a tendency reported for the left testicle to be larger than the right testicle (**Rua et al., 2017**), suggesting that this occurs due to the earlier development of the left testicle in relation to the right testicle. **Tibary et al. (2005)** reported difficulties in approaching horses from the right side.

Historically the breeding season in the northern hemisphere extends from 15 February to 15 July (**Allen and Wilsher, 2011**), nowadays it is unusual stallions in Algeria to mate later than mid-June. We found that April and May were the best months for stallions breeding with higher DSP, DSO, and other testicular measurements. **Turner (2019)** showed that in breeds subjected to artificial insemination, semen cryopreservation is indicated for a genetically valuable stallion at the pinnacle of its fertility.

Correlations coefficients (Table 3) revealed that various measurements of the testis correlated with each other ($P < 0.01$). These results were in agreement with those reported by **Tibary et al. (2005); Samper et al. (2007)** and **Rua et al. (2017)**. The scrotal width (TSW) correlated significantly with all testicular measurements and the age of stallions ($p < 0.01$). Stallions are seasonal breeders and this seasonal variation in testicular size is greater in older stallions than in younger stallions (**Houssou et al. 2020**).

The widths of both the right and left testicles are very correlated between their ($r=0.85$, $p < 0.01$). According to **Tibary et al. (2005)**, the number of sperm cells that a stallion can produce is an indicating parameter to estimate its fertility, it depends on the quantity of the functional testicular and determines the number of mares mating by a stallion.

CONCLUSION

The study undertaken over a year allowed us to assert the strong correlation between the scrotal width with the various testicular parameters to place the period the most suited to the reproduction under our latitudes during April and May. In addition, this study allowed us to confirm that the breed factor had a significant influence on the testicular parameters under the Algerian conditions and allowed us to plan a national program of artificial insemination.

Conflict of interest: The authors declare that there is no conflict of interest in this study.

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