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Measurement of the reproductive efficiency of Arabian stallions intended for AI in Algeria

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ABSTRACT

The aim of this study was to determine the reproductive efficiency of Arabian stallions presented at CNIAAG and selected for an artificial insemination (AI) program. Ten Arabian stallions between 8 and 15 years of age were subjected to an analysis of the reproductive parameters. Assessment of sexual behaviour, testicular measurements and appreciation of semen quality collected with the help of artificial vagina was done. There was a significant correlation between the sexual behaviour, the spermatic parameters and the testicular parameters, especially between the number of mounts with the motility and the daily sperm ejaculated (DSP) (r=0.99). The testicular volume total and mounts was highly correlated to the average volume of ejaculate (73.33 ± 60.27 ml) and total Sperm concentration (billions) (r=0.99) which allowed us to produce 38 straws intended for the preservation. Based on the results, it is concluded that there is a positive correlation between (TSW) and motility, the various measurements of testis size were highly correlated with each other; and consequently to predict the fertility of the stallions from the testicular measurements.

Keywords: Algeria, Arabian, Reproductive efficiency, Stallion

The stallions are selected for breeding, primarily based on their athletic powers, or other phenotypic characteristics. Fertility or fertility potential are usually at best secondary considerations (El-Badry *et al.* 2016, Houssou *et al.* 2018). To achieve maximum reproductive efficiency in a stallion breeding stud, it is important that the stallions are free of genital abnormalities and free of infectious agents that can affect the genital tract that damage the normal reproductive processes.

In Algeria, horse breeding is a sector that maintains its economic importance. Horses with good phenotypic and genetic characteristics are bought for large amounts of money around the world. For the preservation and distribution of the Arabian horses, an AI program was set up at the Artificial Insemination and Genetic Development Center (CNIAAG). The choice of breeding stallions outside the performances for which they were selected necessarily passes through the knowledge and appreciation of their sexual function. Indeed, the latter is not dependent on the results of the semen evaluation alone but requires further complementary examinations.

There is lack of information about the characteristics associated to reproductive efficiency as the testis biometry and sexual behaviour of the Algerian stallions; therefore, it needs more research, especially for selection of the stallions with highest fertility potential. The genetic variability and its relation to the semen characteristics could explain the differences in the fertility (Alamaary *et al.* 2019, Houssou *et al.* 2020). One of the most important problems in horse breeding is the prediction of stallion fertility. Seminal and behavioural parameters routinely used to access and predict stallion fertility have a limited capacity for general infertility detection (Gamboa and Ramalho-Santos 2005).

The true indices of fertility are the pregnancy and foaling rates, however both are retrospective and are influenced dramatically by factors extrinsic to the stallion, such as mare quality and breeding management (Sullivan *et al.* 1975, van Buiten *et al.* 1998, Morris and Allen 2002, Houssou *et al.* 2018).

Belkadi *et al.* (2017) reported that the typical method of evaluating the fertility of male breeding is the examination of sperm production. Therefore, more consideration is required for semen extender to improve the frozen semen quality for horses (Alamaary *et al.* 2019).

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 by the following: clinical examination, testicular

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measurements in order to assess the daily sperm output, assessment of sexual behaviour and to find their correlation with the seminal quality.

MATERIALS AND METHODS

Animals and location: The study was carried out at Artificial Insemination and Genetic Development Center (CNIAAG). This center is located near the National Stud on the level of Wilaya of Tiaret at an altitude of 1,086 meters, a latitude of 35°15′N and a longitude of 1°26′E. The geographical situation characterizes this region by a semi-arid climate.

Clinical examination of the genital tract: Ten healthy Arabian stallions between 8 and 15 years were presented to examine the sexual behaviour, testicular measurement and semen analysis during breeding season (June 2015). A thorough physical examination of external genital organs always is essential for prediction of stallion fertility (Blanchard *et al.* 2011). The body condition score (BCS) was evaluated on a scale of 1 to 9 as suggested by Henneke *et al.* (1983).

Testicular parameters: After the clinical examination, the testis were measured with a calliper and the following parameters were recorded: total scrotal width (TSW; cm); left testicular width (LTW; cm); right testicular width (RTW; cm); left testicular length (LTL; cm); right testicular length (RTL; cm); left testicular height (LTH; cm) and right testicular height (RTH; cm).

Testicular weight (TW); albuginea weight (AW), testicular parenchyma weight (TPW), daily sperm output (DSO), daily sperm product (DSP) and total testicular volume (TTV) were calculated as per the formulas given in Table 1.

Sexual behaviour: The sexual behaviour of stallions for artificial insemination (AI) is not different from that observed during in-hand natural service (NS) (Noue *et al.* 2001). The sexual behaviour sequences have been described in detail in Cavinder *et al.* (2010), Rua *et al.* (2015), Waheed *et al.* (2015), McDonnell (2016), Houssou (2016), Zeidan *et al.* (2017) and Houssou *et al.* (2020).

Semen collection and processing: Semen was collected at a rate of one ejaculate per day per stallion. Collection was achieved using an artificial vagina (Missouri model) on a dummy in the presence of a teaser mare displaying estrous behaviour. The artificial vagina was lubricated with a non spermicide lubricant (Génia. Fr). Total semen volume was directly read on the collector tube. Immediately after collection, the gel fraction of the ejaculate was removed. Semen was filtered through sterile gauze, and ejaculate volume and colour were determined.

The gel-free semen was maintained at 37°C in a water-bath. Filtered semen of each ejaculate was diluted at a ratio of (1:1) during the pre-dilution with extender (ref. 13565/0001) and semen analyses to assess sperm quality. Progressive motility was estimated visually under light microscopy at 37°C on a heated stage with a scale of 1 to 5. In accordance with Barrier *et al.* (2016) and WBFSH recommendations (2020), semen was classified acceptable for conservation when progressive motility after thawing was >35%.

Total sperm concentration (billions) was measured using a previously calibrated photocolorimeter at 530 nm. The diluted semen was centrifuged at 500 g for 5 min, the supernatant was aspirated, and the pellet was resuspended at a ratio of 1:4 with the final extender (equiplus one step) is added at a ratio of (1:4), that allowed us to condition 38 straws of 0.5 ml. Straws were placed on a rack into the freezing chamber of a computer-controlled rate freezer at 8°C (Ice-Cube computer controlled rate freezer 14 S). Semen was first cooled to 5°C at a cooling rate of 0.3°C/min, subsequently within 3 min to –25°C (10°C/min) and finally to –140°C at a cooling rate of 25°C/min. Straws were removed from the freezing chamber and plunged directly into liquid nitrogen at –196°C.

Statistical analysis: Data were analyzed using IBM SPSS 20 and expressed as the mean±standard error of mean (SD) min; max and variance. The Pearson's correlation coefficients were used to assess the association between the parameters studies.

RESULTS AND DISCUSSION

During this study, of the ten stallions presented, two rejected the dummy, and on examination of the eight ejaculate collected, five samples presented only seminal plasma. Therefore, only three ejaculates were analyzed. Pickett (1993) reported that sexual excitation increases the secretion of the seminal vesicles, increasing the volume and proportionately decreasing the concentration**.**

Table 1. Testicular parameters estimated by testicular measurement

Testicular parameter	Formulas
Testicular weight (g)	TW = 71 (Total scrotal width)−140
Albuginea weight	AW = 0.145 × (weight calculated)+2.52
Testicular parenchyma weight	TPW = TW−AT
DSO (Total scrotal width in mm)	$(0.066\times TSW-3.36)\times 10^9$
DSP (Total scrotal width in mm)	$(0.093\times TSW-4.88)\times 10^9$
Testicular volume right or left (ml)	TV = 0.5233 × lenght/2 × width/2 × height/2
Total testicular volume	TTV = TVL+TVR

Source: Blanchard *et al.* (2011), Rajak *et al.* (2013), Waddington *et al.* (2017), Perumal *et al.* (2017), Rua *et al.* (2017) and Papa *et al.* (2020).

Testicular measurements: The left and right testicular width were 4.53±1.05 cm and 4.48±0.89 cm respectively; left and right testicular height were 5.83±0.63 and 5.30±0.34 cm respectively and the left and right testicular length were 7.07±0.92 and 6.94±0.86 cm respectively. Whereas, the total width of the scrotum corresponding to the largest width including both testicles and the scrotal envelope was 9.00±0.42 cm. Our results are in agreement with the results reported by Blanchard *et al.* (2011) and Ponthier *et al.* (2012).

In context to testicular biometry, there were differences between left and right testicle. The tendency for the left testicle to be large than the right testicle has been reported by Borges *et al.* (2010), Hafez and Hafez (2004) and Rua *et al.* (2017). Similarly, Hafez and Hafez (2004) observed the same, suggesting that this occurs due to the earlier development of the left testicle in relation to the right testicle.The average value of daily sperm product (DSP; 3.62±0.17 10^9), daily sperm output (DSO; 2.58±0.27 10^9) were lower as compared to that reported by Amann *et al.* (1993), Dadoune *et al.* (2001) and Tibary *et al. (*2005). According to Pickett (1993) and Tibary *et al.* (2005), the number of the sperm cells which a stallion can produce is an indicating parameter to estimate its fertility, and it depends on the quantity of the functional testicular, and determines the number of mares minting by a stallion (Table 2).

Seminal parameters and sexual behaviour: The time of semen collection of stallions was less than 200 sec which was in agreement with that reported by Noue *et al*. (2001) and Najjar *et al.* (2010). Similarly, the number of mounts (1.33±0.57) was also in agreement with those reported by Noue *et al.* (2001), Najjar *et al.* (2010) and Houssou *et al.* (2020).

The results recorded during the study are comparable with those observed by Hafez and Hafez (2004), Pesch *et al.* 2006 and Tibary *et al.* 2005. There was some variation in total sperm concentration (billions), progressive motility, and semen volume between stallions. Dowsett and Knott (1996) reported that the Arabian Purebred demonstrated higher total harvested volume (with reduced gel fraction), and high total sperm concentration (with high rate of viability), as compared to the Thoroughbred (Dowsett and Knott 1996, Blanchard *et al.* 2011). Different studies have indicated the influence of age of the stallion on sperm production and quality (Talluri *et al.* 2017). Sexual maturity in horses over the age of 5 years varies as reported by Ortega-Ferrusola *et al.* (2014). However, all the stallions in our study were mature (≥8 years). In the present study, we used the sperm motility as the primary criterion to evaluate the stallion semen quality. This technique was also used by other authors (Love *et al.* 2015, Battut *et al.* 2017, Aurich *et al.* 2020). The percentage of ejaculates acceptable for artificial insemination (AI) after cryopreservation only (30%) in our study was lower than average reported on Arab stallion by Aurich *et al.* (2020).

Barrier *et al.* (2016) who reported a high correlation between fertility and motility recommend a minimum 35% progressive motility.

Rodenas *et al*. (2014) reported that spermatozoa subjected to cryopreservation are very sensitive to a rapid reduction in temperature from room temperature to 5°C, which produces cold shock. Freezing-induced dehydration of the cells causes a more severe phase transition to a highly

Table 2. Morphometric testicular and testicular parameters in Arabian stallion

	Age	BCS	RTW (cm)	LTW (cm)	TSW (cm)	RTL (cm)	LTL (cm)	RTH (cm)	LTH (cm)	TW (g)	AW (g)	TPW (g)	DSO ($\times10^9$)	DSP ($\times10^9$)	TTV (mm^3)
Mean	12.66	5.16	4.48	4.53	9.00	6.94	7.07	5.30	5.83	498.00	74.87	424.00	2.58	3.62	287.70
SD	4.04	0.35	0.89	1.05	0.42	0.86	0.92	0.34	0.63	29.88	4.32	25.59	0.27	0.17	11.02
Min	8.00	4.00	3.83	3.86	8.52	6.20	6.15	5.01	5.11	465.00	69.89	394.00	2.26	3.43	278.01
Max	15.00	7.00	5.49	5.76	9.27	7.90	8.01	5.68	6.32	518.00	77.74	441.00	2.77	3.76	299.70

Total scrotal width (TSW); left testicular width (LTW); right testicular width (RTW); left testicular length (LTL); right testicular length (RTL); left testicular height (LTH) and right testicular height (RTH). Testicular weight (TW); albuginea weight (AW); testicular parenchyma weight (TPW); daily sperm output (DSO); daily sperm product (DSP); and total testicular volume (TTV).

Table 3. Sexual behaviour and seminal characteristics of Arabian stallion

	Age	Semen volume (ml)	Gel-free semen (ml)	Total sperm per ejaculate ($\times10^9$)	Motility	Preparation time (sec)	Number of mounts	Collection time (sec)
Mean	12.66	73.33	30.00	3.33	3.66	101.00	1.33	161.00
SD	4.04	60.27	26.45	1.53	0.28	18.05	0.57	72.02
Min	8.00	10.00	10.00	2.00	3.50	21.00	1.00	82.00
Max	15.00	130.00	60.00	5.00	4.00	70.00	2.00	223.00

ordered gel phase resulting in a loss of selective permeability and integrity of the plasma membrane (Oldenhof *et al.* 2012), leading to loss of motility and diminished metabolism. Stallion seminal plasma has an effect on the function and survival of ejaculated spermatozoa during *in vitro* storage and in the female genital tract, and to some extent individual stallion variation in the freezability of the semen (Talluri *et al.* 2017).

Correlation test: Correlation coefficients (Table 4) showed that various measurements of the testis were 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) and the daily sperm product were predicted from the scrotal width testicular (Table 4).

In this study, 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 are in agreement with those reported by Tibary *et al.* (2005), Samper *et al.* (2007), Hafez and Hafez (2004), Rua *et al.* (2017).

The correlation reveals that the width of both right and left testicles are very correlated (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 was (TW) 501.40±74.70 g.

Tibary *et al.* (2005), Blanchard *et al.* (2011), Samper *et al.* (2007) reported that the total width of the scrotum (TWS) measurement is highly correlated with the daily sperm production (DSP) and sperm output (DSO) and also with the weight of the testicular parenchyma which is in accordance with our result (r=1, p<0.05 et p>0.01).

A significant correlation existed between the preparation time of the stallions during the sperm collection, with the daily sperm production (DSO) and the motility (r=0.99; p <0.05). Similar was the case between the number of mounts and motility (r = 0.99, p <0.05). Najjar *et al.* (2010) found that the number of mounts and the collection time affected the fertility of fresh semen. Clément *et al.* (1995) showed that there was not any determined relation between fertility and sexual behaviour. Anand and Yadav (2016) reported that motility is a phenomenon responsible for transport of spermatozoa from site of deposition to site of fertilization. This process peculiar to spermatozoa depends on the physiological and morphological status of a sperm cell and is highly sensitive to stressor encountered during the process of cryopreservation. Clément (1995) reported that the concentration, total sperm concentration and % of motility spermatozoa at 0 h are more often correlated with fertility.

Present study was undertaken to demonstrate that the choice of breeding stallions in Algeria begins with the clinical examination of the reproductive system, the estimation of the daily sperm production, daily sperm output following testicular measurements, evaluation of sexual behaviour and a spermogram selected for an artificial insemination program. A significant correlation between the different criteria measured; lead us to conclude that the

Table 4. Coefficient of correlation among morphometric testicular, sexual behaviour and seminal characteristics in Arabian stallion

r	TSW (cm)	TW (g)	TPW (g)	DSO ($\times10^9$)	DSP ($\times10^9$)	TTV (mm^3)	Semen volume (ml)	Total sperm per ejaculate ($\times10^9$)	Motility	Preparation time (sec)	Number of mounts	Collection time (sec)
TSW (cm)	1	1.00**	1.00**	1.00*	0.98	0.81	0.81	0.81	0.57	0.99**	0.57	0.97
TW (g)		1	1.000**	1.00*	0.98	0.81	0.81	0.81	0.57	0.99**	0.57	0.97
TPW (g)			1	1.00*	0.98	0.81	0.81	0.81	0.57	0.99**	0.57	0.97
DSO ($\times10^9$)				1	0.99	0.82	0.82	0.82	0.59	0.99*	0.59	0.97
DSP ($\times10^9$)					1	0.89	0.89	0.88	0.68	0.98	0.68	0.99
TTV (mm^3)						1	0.99**	0.99**	0.94	0.8	0.94	0.92
Semen volume (ml)							1	1.00**	0.94	0.8	0.94	0.92
Total sperm per ejaculate ($\times10^9$)								1	0.94	0.80	0.94	0.92
Motility									1	0.56	0.99**	0.74
Preparation time (sec)										1	0.57	0.57
Number of mounts											1	0.57
Collection time (sec)												1

**, correlation significant at 0.01; *, correlation significant 0.05; Total scrotal width (TSW); Testicular weight (TW); Testicular parenchyma weight (TPW); Daily sperm output (DSO109); Daily sperm product (DSP109) and Total testicular volume (TTV); seconds (sec).

evaluation of the testis size is very important in stallions' selection, since it is an indirect determinant of their reproductive potential.

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