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Relationship of serum testosterone, sperm production, and testis traits with fertility of Mangalarga Marchador stallions¤

Relación entre testosterona sérica, producción espermática y características testiculares con la fertilidad en sementales Mangalarga Marchador

Relação entre testosterona sérica, produção espermática e características testiculares com fertilidade em garanhões Mangalarga Marchador

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Abstract

Background: Assessing breeding soundness is important to predict the potential fertility of stallions. **Objective:** To investigate the association of testis traits, total number of spermatozoa, and testosterone with fertility of Mangalarga Marchador breed stallions. **Methods:** The traits measured were testicular length, width and height, right and left testicular volume and total testicular volume. We also evaluated the total number of spermatozoa (TNS), serum testosterone concentration and fertility of stallions (by embryo recovery from donor mares) in the breeding and non-breeding seasons during 1 year. Analysis of variance was performed to verify the effects of age category (AC; young/adult) and reproductive season on the traits. Correlations and repeatability of the traits were also calculated. **Results:** The AC did not influence testis traits ($p>0.05$). Reproduction season affected left testicular length, left testicular height, right testicular width and height ($p<0.05$). The AC and season affected testis volume ($p<0.05$). There were no effects of AC and season on TNS and testosterone ($p>0.05$). We observed strong correlations between all testicular measurements and testicular volume (0.53 to 0.75), medium correlation between TNS and testis volume (0.32), and low correlation between testosterone and testis traits (0.20). Repeatability of the characteristics of left and right testis was medium to high (0.22 to 0.78). Embryo recovery rate was 60% and its correlation with TNS was 0.44. **Conclusion:** Testis size evaluation is recommended over the

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evaluation of serum testosterone concentration to predict sperm output. Sperm output (TNS) is a good predictor of stallion fertility.

Keywords: *equine, hormone, male, reproduction, testis size.*

Resumen

Antecedentes: Es importante realizar examen andrológico de sementales para predecir su potencial reproductivo. **Objetivo:** Evaluar la asociación entre características testiculares, número total de espermatozoides y testosterona con la fertilidad de sementales de raza Mangalarga Marchador. **Métodos:** Las características medidas fueron: longitud testicular, ancho y altura del testículo. También fue calculado el volumen testicular derecho, izquierdo y total, así como el número total de espermatozoides (TNS), concentración sérica de testosterona y fertilidad (por recuperación embrionaria de las yeguas) de los sementales dentro y fuera de la temporada reproductiva durante un año. Se realizó análisis de varianza para evaluar el efecto de categoría de edad (AC) y estación reproductiva sobre las características. **Resultados:** La AC no influenció las características testiculares (p>0,05). La estación reproductiva influenció la longitud del testículo izquierdo, la altura del testículo izquierdo, y el ancho y altura del testículo derecho (p<0,05). La AC y la estación reproductiva influenciaron el volumen testicular (p<0,05). No hubo efecto de AC y estación reproductiva en el TNS y testosterona (p>0,05). Se observó una alta correlación entre todas las características testiculares y el volumen testicular (0,53 a 0,75), mediana correlación entre TNS y el volumen testicular (0,32), y una baja correlación entre las medidas testiculares y la testosterona (0,20). La repetitividad de las medidas testiculares fue media a alta (0,22 a 0,78). La tasa de recuperación embrionaria fue de 60% y la correlación con TNS fue 0,44. **Conclusión:** Para predecir la producción espermática se recomienda evaluar las características testiculares, sin necesidad de medir testosterona. La producción espermática es un buen predictor de la fertilidad de los sementales.

Palabras clave: *equino, hormona, macho, reproducción, tamaño testicular.*

Resumo

Antecedentes: Realizar o exame andrológico no garanhão, é importante para predizer seu potencial reprodutivo. **Objetivo:** Avaliar a associação das características testiculares, número total de espermatozoides, testosterona e fertilidade de garanhões Mangalarga Marchador. **Métodos:** As características mensuradas foram: comprimento do testículo, largura do testículo e altura do testículo. Também foi calculado o volume testicular, direito, esquerdo e total, assim como calculado o número total de espermatozoides (TNS), concentração sérica de testosterona e fertilidade dos garanhões (pela recuperação embrionária das éguas doadoras) durante e fora da estação reprodutiva durante um ano. Foi feita análise de variância para verificar efeito da categoria de idade (AC) e estação reprodutiva sobre as características. **Resultados:** A AC não influenciou nas características testiculares (p>0,05). Estação reprodutiva influenciou no comprimento testicular esquerdo, altura testicular esquerda, largura e altura testicular direita (p<0,05). A AC e estação reprodutiva influenciaram no volume testicular (p<0,05). Não houve efeito da AC e estação reprodutiva no TNS e testosterona (p>0,05). Foi observado alta correlação entre todas características testiculares e volume testicular (0,53 a 0,75), media correlação entre TNS e volume testicular (0,32), e baixa correlação entre medidas testiculares e testosterona (0,20). A repetitividade das medidas testiculares foi meia a altas (0,22-0,78). A taxa de recuperação embrionária foi 60% e a correlação com TNS foi de 0,44. **Conclusão:** É recomendada a avaliação das características testiculares para predizer a produção espermática sem necessidade de mensurar testosterona. A produção espermática é um bom preditor da fertilidade dos garanhões.

Palavras chave: *equino, hormônio, macho, reprodução, tamanho testicular.*

Introduction

The assessment of reproductive traits of stallions, such as semen quality and testicular parenchyma is important to predict their reproductive efficiency (Love, 2014). Testicles are the male gonads, where the production of sperm occurs. Testes in stallions are ovoid, slightly compressed on both sides, arranged in an almost horizontal position (Hafez and Hafez, 2004). Testicular size can change during lifetime and in different breeding seasons. Furthermore, age and breeding season can affect testicular size by increasing the number of somatic and germ cells present in the testicular parenchyma. This parenchyma consists of seminiferous tubules and interstitial tissue (McKinnon *et al.*, 2011).

Testicular volume and size are directly related with the number of sperm produced (Chenier, 2007; McKinnon *et al.*, 2011), making evaluation of testicle measurements very important to predict the sperm output of a stallion used in a breeding season. These evaluations are also important since semen quality is crucial to the success of embryo transfer programs.

However, little information is available regarding reproductive efficiency of stallions in Brazil. In the north of Rio de Janeiro state, southeastern Brazil, there are many horse breeders and stud farms that use reproductive biotechnologies like artificial insemination and embryo transfer. However, there few studies have been conducted to evaluate testis size and its association with sperm production and fertility of Mangalarga Marchador stallions between different seasons (Freitas, 2010; de Oliveira, 2014). The objectives of this study were to verify the associations of serum testosterone concentration, testis size, sperm production and stallion fertility as well as to calculate the repeatability and phenotypic correlations of these traits in Mangalarga Marchador stallions.

Materials and methods

Ethical considerations

This experiment was approved by the committee on ethical use of animals (CEUA–UENF) in 2013, according to the standards of the Brazilian Society of Laboratory Animal Science/Brazilian College of Animal Experimentation (SBCAL/COBEA), under protocol number 245.

Study design

We evaluated fifteen (n = 15) Mangalarga Marchador stallions (weight: 400.1 ± 40.3 Kg), separated into two age categories (AC): Young (2.5 to 4 years old) and adults (≥ 5 years old). The animals were located in a stud farm in northern Rio de Janeiro state (latitude 21° 45' 15" South, longitude 41° 19' 28" West, and 13 m.a.s.l). The average monthly daylight hours in the region are 13.2, 12.8, 12.2 and 11.6 (from January to April) and 10.8, 10.9, 11.3 and 12.0 (from June to September) (EMBRAPA, 2013; INMET, 2013).

The evaluations were conducted during the breeding season (BS) and non-breeding season (NBS) in 2013. In the north of Rio de Janeiro, the BS occurs during spring and summer, from September until April, and the non-BS occurs during fall and winter, from May to August.

During the experimental period, the stallions were maintained in individual stalls with 16 m^2 floor space. Stallions were fed commercial pelleted feed (Equitec®, São Paulo, SP, Brazil) with 12% crude protein, 15% fibrous fraction and 20 g/Kg fats (1.5% of live weight). They were also fed hay three times per day (1.5% of live weight) and had *ad libitum* access to water. All stallions maintained good body condition during the experimental period.

We performed evaluations of testis size (15 stallions) during the BS (January, February, March and April) and during the NBS (June, July, August and September; total of 120 observations). Testis size of each stallion was measured eight times with intervals of 30 d in each reproduction season.

Testis measurements and volume

The left and right testicular lengths (LTL and RTL) were measured from the cranial to caudal edge, the left and right testicular widths (LTW and RTW) were measured from lateral sides of each testis, and the left and right testicular heights (LTH and RTH) were measured from the ventral dorsum of each testis. All measurements were performed with a caliper (in centimeters). Testicular volume (TV) was calculated using the formula suggested by Love *et al.* (1991):

$$TV_{L\ or\ R} = 4/3\ \pi\ (TL/2 \times TW/2 \times TH/2)$$

Where:

π: 3.14.

TL: Testicular length.

TW: Testicular width.

TH: Testicular height.

Total testicular volume was calculated as TTV: $TV_L + TV_R$.

Semen analysis

Semen was collected with an artificial vagina (Botupharma®, Botucatu, SP, Brazil) eight times from each stallion during the BS (January, February, March and April) and NBS (June, July, August, and September) with 30-d intervals. Sperm concentration ($\times 10^6$ sperm/mL) was assessed by counting spermatozoa in a Neubauer chamber (New Optics, Hachtsebaan, Keerbergen, Belgium) using optical microscopy (Zeiss Axiovert 35; Carl Zeiss Inc., Thornwood, NY, USA) at 40X. Semen volume was evaluated in graduated tubes (Falcon Plastics, Los Angeles, CA, USA; 50 mL). Furthermore, we calculated total number of spermatozoa (TNS $\times 10^9$ sperm/ejaculate) through TNS = sperm concentration x semen volume.

Testosterone analysis

Serum testosterone concentration was determined from blood samples collected from each stallion eight times (four times each season) during the morning —after semen collection— using tubes (Vacutainer® 130, BD Diagnostics, Treton, New Jersey, USA) without ethylenediaminetetraacetic acid (EDTA). The samples were collected during both the BS and NBS. The blood was transported (40 min) to the laboratory in a box (Styrofoam®, Trenton, New Jersey, USA) on ice. The tubes with blood samples were centrifuged at 1,400 x *g* for 15 min. The serum was separated, and the samples stored in a freezer at -20 °C until analysis. The serum testosterone concentrations were determined through a solid phase radioimmunoassay (RIA) technique using a commercial diagnostic kit (Immunotech Beckman, Coulter Laboratories, Marseille, France). Sample extraction and dosing were performed according to the manufacturer's instructions.

Evaluation of stallion fertility

Fertility evaluation involved 525 assessments of embryo recovery rates in donor mares during the BS and NBS. The mares were inseminated with fresh semen from the stallions and embryo recovery was performed 8 d after ovulation. All donor mares used in this experiment were adult with an average age of 14 years (range 10 to 24 years old). All mares underwent reproductive soundness examination and presented good fertility conditions (no problems related to the reproductive tract). Mares used as embryo donors did not present any symptomology of endometritis, such as uterine fluid and neutrophils during cytology.

The protocol to induce ovulation in donor mares was 1 mg/mL of desloreline (Sincrorelin®, Ourofino, São Paulo, SP, Brazil) when a 35 mm follicle was detected with ultrasound. All donor mares were fed commercial pelleted feed (Equitec®, São Paulo, SP, Brazil) with 12% crude protein, 15% fibrous fraction and 20 g/Kg fats (1.0% of live weigh). They were also fed hay three times per day (2.0% of live weight) and had *ad libitum* access to water during the BS.

Donor mares continued cycling during the NBS because of the favorable environmental conditions in the North of Rio de Janeiro state. Though, nutrition changed this period by feeding donor mares the same pelleted feed as in BS, but at 2.0% of live weight. They were also fed hay three times per day (2.0% of the mares live weight) and had *ad libitum* access to water.

Statistical analysis

Analysis of variance of testicular traits was performed to verify the effects of age category of stallions (AC) and reproductive season (RS) on the testicular traits, total number of spermatozoa and serum testosterone concentration (PROC MIXED, SAS®, version 9.4, SAS Inst., Cary, NC, USA). The means were compared using the Student-Newman-Keuls (NTK) test at 5% probability. The final model was:

$$Y_{ijk} = \mu + AC_i + RS_j + e_{ijk}.$$

Where:

Y_{ijk}: Dependent variable.

μ: Overall mean, associated with the dependent variable.

AC_i: Effect of the i^{th} age category.

RS_j: Effect of the j^{th} reproductive season.

e_{ijk}: Random residual associated with each observation.

The CORR procedure of the SAS (Software version 9.4, SAS Institute Inc., Cary, NC, USA) program was used to calculate the pairwise correlations between traits. Repeatability of the testis traits was calculated from the estimates of variances by REML using the VARCOMP procedure (SAS, 2009), where repeatability (t) = variance (stallion)/[variance (stallion) + variance (residual)]. Embryo recovery rate was analyzed by frequency (proc freq; SAS, 2009).

Results

Table 1 shows the means and standard errors of the testis traits in relation to AC and RS. In general, the means of testis measurement traits were higher in the BS than in the NBS, except LTW and RTL, which did not show difference between both seasons.

The AC did not affect testis traits LTL, LTH, RTL, RTH nor RTW ($p>0.05$). However, we observed an effect of AC on LTW ($p<0.05$). The average testicular measurements of Mangalarga Marchador stallions were 8.6 ± 0.8 cm for LTL, 5.4 ± 0.8 cm for LTW, 6.1 ± 0.9 cm for LTH, 7.8 ± 1.6 cm for RTL, 5.7 ± 0.9 cm for RTW, and 6.3 ± 0.8 cm for RTH.

The AC and the RS affected the testis volume ($p<0.05$). The average testis volumes were 163.6 ± 49.6 cm^3 for LTV, 150.0 ± 44.8 cm^3 for RTV, and 318.1 ± 83.8 cm^3 for TTV.

The means of RTV and TTV were different between young and adult stallions. However, there was no variation in LTV between young and adult stallions. In general, all variables of testicular volume varied between both seasons. The highest means were in adults and during the BS (Table 2). The means of TNS and serum testosterone concentration did not vary between AC and seasons.

Table 1. Means and standard errors of testis traits in relation to age category (AC) and reproduction season (RS) in Mangalarga Marchador stallions.

Effect	LTL (cm)	LTW (cm)	LTH (cm)	RTL (cm)	RTW (cm)	RTH (cm)
Age category (AC)						
Young	8.4 ± 1.1[a]	5.3 ± 1.0[b]	5.9 ± 1.0[a]	7.3 ± 1.8[a]	5.6 ± 1.6[a]	6.0 ± 0.9[a]
Adult	8.7 ± 0.8[a]	5.9 ± 0.7[a]	6.3 ± 1.0[a]	8.1 ± 1.6[a]	6.0 ± 0.9[a]	6.5 ± 1.0[a]
Reproduction season (RS)						
BS	8.9 ± 0.8[a]	5.7 ± 0.9[a]	6.4 ± 0.9[a]	8.0 ± 1.7[a]	6.1 ± 1.2[a]	6.6 ± 1.0[a]
NBS	7.6 ± 0.9[b]	5.3 ± 0.9[a]	5.1 ± 0.7[b]	7.3 ± 1.6[a]	4.9 ± 1.0[b]	5.3 ± 0.7[b]

Means followed by the same superscript letter within columns are not significantly different ($p>0.05$). Left and right testicular length (LTL and RTL); left and right testicular width (LTW and RTW); left and right testicular height (LTH and RTH).

Table 2. Means and standard errors of testis traits, sperm production, and serum testosterone concentration in Mangalarga Marchador stallions in relation to age category and reproduction season.

Effect	LTV (cm^3)	RTV (cm^3)	TTV (cm^3)	TNS (× 10^9)	Testosterone
Age category (AC)					
Young	144.6 ± 53.4[a]	131.9 ± 48.9[b]	276.6 ± 90.6[b]	13.3 ± 11.4[a]	0.80 ± 0.31[a]
Adult	173.9 ± 51.8[a]	165.7 ± 49.1[a]	339.6 ± 97.1[a]	14.0 ± 12.6[a]	0.55 ± 0.26[a]
Reproduction season (RS)					
BS	176.0 ± 50.6[a]	163.3 ± 46.0[a]	340.0 ± 90.8[a]	14.6 ± 12.9[a]	0.67 ± 0.38[a]
NBS	115.5 ± 39.1[b]	106.7 ± 45.9[b]	233.6 ± 69.5[b]	10.2 ± 7.0[a]	0.64 ± 0.21[a]

Means followed by the same superscript letter within columns are not significantly different ($p>0.05$). Left and right testicular volume (LTV and RTV), total testicular volume (TTV), and total number of spermatozoa (TNS). Breeding season (BS) and non-breeding season (NBS).

Table 3 shows pairwise correlations of age category, testis traits, TNS and testosterone. Age category presented high correlation with LTL, LTV, RTV, and TTV. In contrast, correlation between age category and RTW was close to zero.

LTL was positively and highly correlated with LTW, LTH, LTV, RTV, and TTV, and the correlations between LTW and the other traits were positive and of medium magnitude, except for testosterone (-0.12). LTW, LTH, LTV, and RTV presented positive and medium to high correlations with all traits, and LTV, RTV and TTV were highly correlated with all traits. Total number of spermatozoa showed medium correlation with TTV (r = 0.32), and testosterone showed low correlation with TTV (r = 0.14) and with TNS (r = 0.20).

Table 4 shows repeatability of testicular traits and testicular volume. Repeatability values were medium to high for all variables.

Table 3. Correlations of age category, testis traits, sperm output, and serum testosterone concentration in Mangalarga Marchador stallions.

	AC	LTL	LTW	LTH	RTL	RTW	RTH	LTV	RTV	TTV	TNS	Test
AC	-											
LTL	0.33*	-										
LTW	0.26	0.53**	-									
LTH	0.29	0.56**	0.1	-								
RTL	0.27	0.15	0.12	0.68*	-							
RTW	0.09	0.34*	0.59*	0.15	0.54*	-						
RTH	0.23	0.39*	0.26	0.61**	0.41*	0.17	-					
LTV	0.42*	0.84**	0.67**	0.76**	0.37*	0.29	0.55**	-				
RTV	0.38*	0.54**	0.39*	0.71**	0.64**	0.20	0.83**	0.74**	-			
TTV	0.42*	0.75**	0.58**	0.79**	0.53**	0.26	0.73**	0.94**	0.92**	-		
TNS	-0.07	0.20	0.28	0.15	0.21	0.04	0.25	0.32*	0.31	0.32*	-	
Test	-0.14	-0.06	-0.12	0.07	-0.02	-0.04	0.10	0.02	0.16	0.14	0.20	-

*Significant (p<0.05), **Highly significant (p<0.001). Age category (AC), left testicular length (LTL), left testicular width (LTW), left testicular height (LTH), right testicular length (RTL), right testicular width (RTW) and right testicular height (RTH), left testicular volume (LTV), right testicular volume (RTV), total testicular volume (TTV), total spermatozoa number (TNS) and testosterone (Test).

Table 4. Repeatability of testicular traits of Mangalarga Marchador stallions.

Traits	Stallion variation	Residual variation	Repeatability
LTL	0.327	0.435	0.45
LTW	0.328	0.435	0.43
LTH	0.343	0.440	0.78
RTL	0.494	0.890	0.36
RTW	0.101	0.367	0.22
RTH	0.214	0.512	0.30
LTV	639.9	659.1	0.50
RTV	884.7	584.8	0.60
TTV	2933.7	1431.9	0.67

Left testicular length (LTL), left testicular width (LTW), left testicular height (LTH), right testicular length (RTL), right testicular width (RTW), right testicular height (RTH), left testicular volume (LTV), right testicular volume (RTV), and total testicular volume (TTV).

A 60% embryo recovery rate was observed from 525 observations during the breeding and non-breeding seasons. The correlation between embryo recovery rate and the TNS was 0.44.

Discussion

The North of Rio de Janeiro state is characterized by hot summers and winter, with average temperature of 25° C and scarce rains during the year (Rua *et al*., 2013). However, the stallions were maintained with plentiful supply of commercial feed, and did not suffer much environmental influence. The average weight of the stallions in this study (400.1 ± 40.3 Kg) was similar to that of other reports. Moura (2010), also studying Mangalarga Marchador stallions, reported that adult animals weight ranges between 400 and 449.2 Kg. Lima *et al*. (2012) observed average Mangalarga Marchador stallion weight of 434.0 ± 25.2 Kg using a particular tape (Ortovet®, São Paulo, SP, Brazil) for equine weight evaluation.

The average testicular traits in the present study were slightly lower than the results reported by Candeias (2010) for testicular measures of Mangalarga Marchador stallions aged 4 to 22 years in Rio de Janeiro state. The likely reason for this difference between studies is that Candeias (2010) only evaluated stallions older than 4 years of age, while we evaluated stallions younger than that. Another factor for the difference is variations of measurements made by different technicians in each experiment.

The difference observed in the testis traits between the BS and the NBS was due to differences of photoperiod between seasons and also to stallion physiology. McKinnon *et al*. (2011) reported that during the breeding season the averages of the testis parenchyma weight, number of Leydig and Sertoli cells and spermatids were higher than observed in the NBS. Despite the small differences in photoperiod during the year in Northern Rio de Janeiro state, it was enough to cause some effect on the testis size. In the BS, the TTV measures were similar to the average observed by Freitas (2010) in Mangalarga Marchador stallions. We observed substantial increase in testicular volume during the BS, which coincides with the period of the year when incidence of light is higher (longer days) in northern Rio de Janeiro state. McKinnon *et al*. (2011) reported that during the BS testis parenchyma weight was due to the increase in the number of Leydig and Sertoli cells and spermatids compared with the NBS.

In contrast to our study, Robalo and Silva *et al.* (2007) in Portugal reported that the testicular volume in adult Lusitano stallions was larger in winter and lower in autumn. The average TTV in adult stallions in our study was higher than the average of TTV reported by Robalo Silva (2007), possibly due to differences between environments and methods of evaluation. Robalo Silva (2007) measured testicular volume using ultrasound and we calculated it using an algorithm (Love *et al*., 1991). Freitas (2010) evaluated testes of Mangalarga Marchador stallions in a colder region than northern Rio de Janeiro and reported that the average of TTV did not vary between seasons of the year.

Although there were variations in testis volume between AC and seasons, these effects were not sufficient to cause changes in total spermatozoa number and serum testosterone concentration (Table 2). Freitas (2010) also observed no differences of TNS and serum testosterone concentration through seasons. Neves (2014) noted no difference of TNS between the BS and NBS. Both authors studied Mangalarga Marchador stallions, but in a different region from the present study. Thus, it can be suggested that Mangalarga Marchador stallions are adapted to different environments because of the small variation in their reproductive traits.

The mean testicular volumes observed in this study in relation to age category corroborate the results presented by Ribas (2006), who observed smaller testes in 4 and 5-year-old than in 7-year-old Pantaneiro stallions in Brazil. According to Roser (2008), growth and maximum testicular development occur in horses after puberty and are characterized by a long process of growth until the stallion reaches 4 to 5 years old. Furthermore, McKinnon *et al*. (2011) reported that percentage of testicular cells and, consequently, testicular volume tend to increase with stallion age. The authors concluded that Leydig cells increase twelve-fold between the ages of 2 and 3 years and fourfold between the ages of 13 and 20 years. Thus, we believe the testis size tends to increase also in adult Mangalarga Marchador stallions.

There was a strong positive correlation between age category and LTL, LTV, RTV, and TTV. This suggests that testes enlarge with age. Mckinnon *et al.* (2011) also observed that testicular size is affected by stallion age. The high positive correlation between LTL and most testis traits indicates that as the length of the testicle increases, other measurements also increase.

All testis volume traits were positive and highly correlated with all other traits. Thus, we suggest that observation of just one of these testicle traits can predict testicular volume. More importantly, we observed that sperm output increased with increase of testis volume. We also observed that stallions with larger testis volume presented slightly higher serum testosterone concentration. Robalo Silva (2007), Freitas (2010) and McKinnon *et al.* (2011) reported a positive correlation between testicular volume and daily sperm output. McKinnon *et al.* (2011) also related that production of testosterone by Leydig cells occurs in the testicular parenchyma and the parenchyma volume is proportional to secretion of testosterone, a hormone involved in spermatogenesis. Thus, we suggest that testis size can be used as a measure to predict sperm production in this breed.

The highest repeatability (Table 4) was LTH ($t = 0.78$). LTV, RTV, and TTV also presented high repeatability ($t = 0.50$, $t = 0.60$ and $t = 0.67$, respectively). Thus, these four measures are more suitable to use as predictors of sperm production capacity, considering there is a high correlation between testicular parenchyma and sperm production (McKinnon *et al.*, 2011). This means it should be possible to predict testicular volume from only one or two measures from each test. All testis measures showed high repeatability, suggesting making only one measurement to evaluate the stallion before the reproductive season.

We observed 60% embryo recovery rate when mares of the same breed were inseminated with fresh semen from stallions during the BS and the NBS. These results are similar to those reported by Uliani *et al.* (2010; 62 and 51% embryo recovery from young and old mares, respectively). Aurich *et al.* (2011) observed 64% recovery rate, and de Paula Lopes *et al.* (2013) reported 72.8% recovery.

All embryo donor mares in this study did not present endometritis symptoms, such as uterine fluid and neutrophils in the uterine cytology, which could characterize failure of the uterine defense mechanism after breeding (Christoffen *et al.*, 2015). So, when mares have a healthy reproductive tract, the success of embryo recovery depends on good semen quality, including spermatozoa concentration and total number of spermatozoa per ejaculate, which determines sperm output.

Since the correlation between TNS and embryo recovery was moderate ($r = 0.44$), we suggest that this semen trait is very important to predict stallion fertility during a reproductive season. Spermatogenesis takes an average of 60 d and during this period some factors can disturb this process, so it is necessary to repeat the breeding soundness examination throughout the reproductive season (McKinnon *et al.*, 2011).

According to the literature, embryo recovery reported in this study is acceptable for healthy stallions (McKinnon *et al.*, 2011; Camargo *et al.*, 2013; Panzani *et al.*, 2014).

We conclude that testis size evaluation is preferable to serum testosterone concentration to predict sperm output, which did not show correlation with total number of spermatozoa. Sperm output (TNS) is a good predictor of stallion fertility and presents good correlation with embryo recovery.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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