Pferdeheilkunde 29 (2013) 3 (Mai/Juni) 335-340

Peri-puberty in male Criollo horses – testicular development, histology of the seminiferous epithelium and epididymal sperm

Joana Weber Gregory[1,2], Anamaria Telles Esmeraldino[3], Lucas Brunelli de Moraes[4], Daniel Vianna Luz[1], Concepta McManus[5], Ricardo Macedo Gregory[1], Rodrigo Costa Mattos[1] and Maria Inês Mascarenhas Jobim[2]

REPROLAB, Veterinary Faculty, Federal University of Rio Grande do Sul (Brazil)[1], Laboratory of Semen and Protein Technology Related to Animal Reproduction, Veterinary Faculty, Federal University of Rio Grande do Sul (Brazil)[2], Veterinary Faculty, Lutheran University of Brazil (Brazil)[3], Institute for Veterinary Research, Animal Health, State Foundation of Agricultural Research (Brazil)[4] and Animal Production Department, Federal University of Rio Grande do Sul (Brazil)[5]

Summary

The objectives of this study were to characterize age-associated changes in testicular size and blood concentrations of testosterone, as well as to evaluate the presence of epididymal sperm. Testicular maturity was evaluated by measuring seminiferous tubule diameter and the presence of the most advanced germ cells in seminiferous epithelium of horses in the peri–puberty period. The animals were surgically castrated and the thorax circumference was taken to estimate body weight. Thirty four male Criollo horses were grouped into four categories: Group I (GI) with four foals aged up to 14 months; Group II (GII) with seven animals over 14 months and less than 17 months; The third group (GIII) included 14 animals over 17 months and less than 19 months; Group (GIV) was composed of nine horses over 19 months and under 34 months of age. After castration, testes were weighed and measured. A segment was collected for subsequent histological evaluation, including measurement of the diameter of seminiferous tubules and the presence of germ cells of the seminiferous epithelium at different stages of development. A blood sample was collected to determine plasma testosterone. The average body weight of the animals increased significantly from 232 kg in GI to 321 kg for horses in GIV. There was no difference between measures from the right and left testes. The testicular weight and volume was greater in animals of GIV and differed significantly compared to younger animals. Plasma levels of testosterone did not differ among age groups. The diameter of seminiferous tubules increased from 89.13 μm in foals of GI to 168.24 μm in horses of GIV. On average, 17.5% of seminiferous tubules had no germ cells in animals from GI, decreasing to 5.4% in GII, 5.2% in GIII and 0.8% in GIV. The number of tubules containing mature spermatids and spermatozoa increased with age. Most significant variations in the increase of testicular volume and diameter of seminiferous tubules were associated with a high presence of spermatozoa in the tubules. In conclusion all animals with 20 months of age or more had reached puberty in the present study and the first spermatozoa appeared in 16 months old colts. Animals presented epididymal sperm when testicular volume and weight were over 16 cm^3 and 23 g, respectively.

Keywords: puberty / horse / testes biometrics / epididymis / spermatogenesis / testosterone / reproduction

Peri-Pubertät beim männlichen Criollo Pferd: Hodenentwicklung, Histologie des Keimepithels und Nebenhodenspermien

Ziel dieser Arbeit war es die altersbezogenen Veränderungen der Hodengröße und des Testosteronspiegels im Blut zu beschreiben und das Vorhandensein von Nebenhodenspermien auszuwerten. Die Hodenreife wurde ausgewertet durch Ausmessen des Durchmessers der Samenkanäle und durch das Vorhandensein der am höchstentwickelten Keimzellen im Keimepithels von Criollo Hengsten in der Phase der Peri-Pubertät. Die Tiere wurden chirurgisch kastriert und der Umfang des Thorax wurde gemessen, um das Gewicht des Tieres zu schätzen. Vierunddreissig männliche Pferde wurden in vier Kategorien eingeteilt: Gruppe I (GI) mit vier Fohlen bis zu 14 Monaten; Gruppe II (GII) mit sieben Tieren die älter als 14 und jünger als 17 Monate waren; die dritte Gruppe (GIII) beinhaltete 14 Tiere im Alter zwischen 17 und 19 Monaten; Gruppe IV (GIV) bestand aus neun Pferden die älter als 19 und jünger als 34 Monate waren. Nach der Kastration wurden die Hoden gewogen und gemessen. Ein Teilstück wurde für anschliessende histologische Auswertungen einbehalten. Die Untersuchungen beinhalteten die Messung des Durchmessers der Samenkanäle und das Vorhandensein von Keimzellen im Keimepithel zu unterschiedlichen Entwicklungsstadien. Eine Blutprobe wurde gesammelt, um Plasmatestosteronkonzentration zu ermitteln. Das durchschnittliche Körpergewicht der Tiere stieg von 232 kg in GI auf 321 kg bei Hengsten in GIV. Es gab kein Unterschied in den Massnahmen zwischen dem rechten und dem linken Hoden. Das Gewicht des Hodens and Volumen war bei Tieren in GIV höher und der Unterschied war signifikant im Vergleich zu den jüngeren Tieren. Die Plasmatestosteronkonzentration unterschied sich nicht zwischen den Altersgruppen. Der Durchmesser des Samenkanals stiegt an von 89.13 μm bei Fohlen in GI auf 168.24 μm bei Hengsten in GIV. Im Durchschnitt hatten 17.5% der Samenkanäle der Tiere in GI keine Keimzellen, dieser Wert fiel auf 5.4% in GII, 5.2% in GIII und 0.8% in GIV. Die Anzahl der Kanäle die reife Spermatiden und Spermatozoiden enthielten, stieg mit zunehmendem Alter an. Die wesentliche Abweichung des Hodenvolumens und Durchmesser der Samenkanäle hingen mit dem hohen Vorhandensein von Spermatozoiden in den Kanälen zusammen. Alle Tiere mit 20 Monaten oder mehr erreichten die Pubertät und die ersten Spermatozoiden erschienen bei 16 Monaten alten Tieren. Ab einer Hodengröße von 16 cm^3 und einem Hodengewicht von 23 g waren in allen Nebenhoden Spermien nachweisbar.

Schlüsselwörter: Pubertät / Pferd / Hoden-Biometrie / Nebenhoden / Spermatogenese / Testosterone / Reproduktion

Introduction

Age related changes in reproduction of male horses have been described in several reports. Spermatozoa in testes were detected one year after birth and the animals take approximately two years to attain sexual maturation (*Swiestra* et al. 1974). The influence of age on testicular size, hormone concentration and puberty has already been studied in horses. Mean age at puberty based on semen characteristics is 21 months, ranging from 14 to 24 months (*Naden* et al. 1990).

Based on the presence of spermatozoa in the epididymis, the puberty is reached between 12 and 16 months (*Wesson* and *Ginther* 1981).

Morphometric analysis on some parameters, such as the number of Leydig and Sertoli cells, and sperm production has also been reported (*Johnson* et al. 1997, *Neves* et al. 2002, *Remiezowicz* et al., 2008, *Figueiró* 2010). The number of Leydig cells per testicular weight increases during the breeding season (*Johnson* and *Thompson* 1987, *Johnson* et al. 1997). Daily sperm production shows the same tendency (*Johnson* and *Thompson* 1987, *Squires* and *Pickett* 2011). A positive correlation between daily sperm output and testicular volume has been demonstrated (*Pickett* et al. 1987, *Love* et al 1991, *Quartuccio* et al. 2011).

Testicular size is an important factor in selecting and managing the stallion for maximum reproductive efficiency (*Squires* and *Pickett* 2011). Otherwise, there is little information of the testicular morphology in horses during peri-puberal development. Studies involving the reproductive characteristics of horses suggest that the influence of breed is significant (*Dowsett* and *Pattie* 1982, *Voss* et al 1982, *Dowsett* and *Pattie* 1987, *Neves* et al. 2005). However, there is no data on testicular development on colts of the Criollo breed before and after puberty. Knowledge of the normal progression of events before and after puberty is necessary if further research is to be conducted on puberty of this breed.

The objective of this study was to determine if testis biometrics, histology, epididymal spermatozoa and plasmatic testosterone concentrations are related to qualitative aspects of spermatogenesis in different age groups.

Materials and Methods

Animals

Data were collected during the summer season (January and March) in the State of Rio Grande do Sul, Brazil. The location of the study was at 30° 16' 57'' latitude south and 55° 53' 47'' longitude west at 145 meters above sea level. The horses were fed exclusively on natural pasture without any supplementation.

Thirty four Criollo spring-born (September-November) colts from commercial herds were divided into 4 age groups as follow: pre-puberal (GI; 12-14 months; n=4); puberal I (GII; 14.1-17 months; n=7); puberal II (GIII; 17.1-19 months; n=14) and post-puberal (GIV; 19.1-34 months; n=9).

The body condition was evaluated, including only animals with conditions equal or more than 3 (1-5). The thorax circumference was measured to estimate the body weight of the animals.

Testicular size

Testes of all horses were surgically removed by a veterinarian using approved animal care practices. Immediately after castration, testicles were separated from the epididymis, weighed, and the length, width and height measured. Testicular volume was determined according to *Love* et al. (1991): 4/3 π (height/2 x width/2 x length/2).

Spermatozoa from epididymis

The epididymis were dissected from the testis. The tail and ductus deferens were isolated and spermatozoa were collected using the retrograde flush technique according to *Bruemmer* (2006), using 5 mL of PBS buffer (pH 7.2). The samples were centrifuged at 1500 xg for 15 minutes to obtain the spermatozoa from the flush of the epididymis. The resulting pellet was transferred to cryovials for storage at -80°C for subsequent laboratory analysis. The presence of spermatozoa from the epididymis tail was analyzed under a light microscope (400X). Animals were considered in puberty when spermatozoa were found in the epididymis (*Wesson* and *Ginther* 1981).

Histological analysis

For histological examination, testicular samples from the 34 horses of different ages were taken with the tunica albuginea, after transversal section. Testicular samples were fixed in 10% phosphate buffered formalin. Samples were dehydrated using alcohol solutions and finally embedded in paraffin. Sections of 5μm slices were stained with hematoxylin-eosin (HE). Methods for evaluation of cell counts and seminiferous tubules were conducted according to procedures previously published (*Abercrombie* 1946, *Moura* and *Erickson* 2001).

To estimate the degree of seminiferous tubule development, 40 tubule cross sections from one testicle were evaluated based on the most advanced germ cell type (*Aguiar* et al. 2006) and registered in one of the following categories: tubules without germ cells, tubules with spermatogonia, spermatocytes, round spermatids, elongate/mature spermatids and spermatozoa in the lumen.

Seminiferous tubules diameters were examined under a conventional light microscope (Olympus CX40,Tokyo, Japan), using a 200X objective, coupled to a digital camera (Olympus C-7070, Tokyo, Japan) connected to a microcomputer. The seminiferous tubules diameters analysis was conducted using the biometric software Motic Images Plus 2.0. Measurements were made basically on 20 circular seminiferous tubules cross sections from each sample.

Testosterone measurements

Single blood samples were obtained immediately before the castration of the horses, placed on ice and returned to the laboratory. Plasma was separated by centrifugation and kept frozen at -20°C until assayed. The analysis of testosterone was carried out using the technique of immune-test of chemiluminescence (Immulite/Immulite 1000®) with values detected starting from 15 ng/dL. Mean intra-assay and inter-assay coefficients of variations for testosterone were 13.0 and 16.4, respectively and the assay sensitivity was 0,1 ng/tube.

Statistical analyzes

Data were analysed using Statistical Analysis Software (SAS®). Analysis of variance (GLM – General Linear Model) was performed to compare (among age groups) the body weight, testicular measurements and weight, testosterone concentration, epididymal sperm, diameter of seminiferous tubules and spermatogenesis. Tukey post hoc test was used to locate differences and $P<0.05$ was regarded as significant. Using the four age groups, Pearson's method was used to estimate the correlations among body weight, testis size and volume, germ cell types numbers, epididymal sperm, diameter of seminiferous tubules and hormone concentrations. Only correlations with P-values <0.05 were considered as significant

Results

At the time of the castration, the average age and body weight of the animals in the four age groups were 13.2±0.6 months and 232±32.2 kg, 16.1±0.1 months and 255±28.7 kg, 17.8±0.3 months and 261±24.7 kg and 26.6±5.4 months and 321±35.5 kg, for GI–GIV respectively. Body weight did not differ among GI, GII and GIII ($P>0.05$), but was higher for horses in GIV in compared to the other groups ($P<0.001$).

Testicular volume and testicular weight increased ($P<0.001$) in GI through the GIII and GIV, but there was no significant difference between testicular weight and testicular volume between GI and GII and between GII and GIII (Fig.1).

Testicle biometry categorized by age groups is shown in Table 1. The analysis of mean testicular measurements for all groups showed no significant differences between the left and right testis (length $P=0.09$, width $P=0.08$ and height $P=0.08$).

Testosterone concentrations were detectable in all animals and mean testosterone concentrations varied from 20 to 129 ng/dL. There was no significant effect of age group ($P=0.42$) or body weight ($P=0.51$).

The development of the seminiferous epithelium of horse as related to the most advanced germ cell type is shown in Table 2. A positive correlation ($R2=0.38$, $P<0.0001$) was observed between the growth of testicular volume and the increase of round spermatids. The raise of mature spermatids is related to the growth of testicular volume ($R2=0.66$, $P=0.0212$), testicular weight ($R2=0.76$, $P=0.0021$) and diameter of seminiferous tubules ($R2=0.60$, $P<0.0001$). Also, the increase of spermatozoa is related to the growth of testicular volume ($R2=0.39$, $P=0.0183$) and diameter of seminiferous tubules ($R2=0.47$, $P=0.0419$). The first spermatozoa in the lumen of the seminiferous tubules appeared in 16 months old colts.

All animals of GIV had spermatozoa present in the epididymis. The first spermatozoa appeared in 16 months old colts. In GIII, twelve horses (85.7%) had sperm stored in epididymal tail, three animals (42.8%) of GII had spermatozoa present in the epididymis and in GI zero colts presented spermatozoa. These parameters were different between age groups

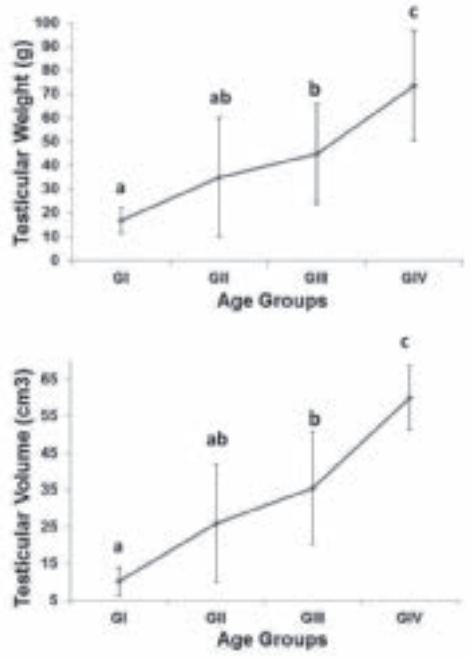

Fig. 1 Mean±S.E. Testicular weight and testicular volume of 34 horses divided into four age groups. [a,b,c] different superscripts indicate statistical difference ($P<0.05$)
Durchschnittswert±S.E. vom Hodengewicht und Hodenvolumen bei 34 Pferden, aufgeteilt in vier Altersgruppen. [a,b,c] Verschiedene obere Zeiger zeigen statistischen Unterschied an ($P<0.05$)

Table 1 Testicle biometry of horses in different age groups / Hoden Biometrie bei Pferden in verschiedenen Altergruppen

	Left testis			Right testis		
Age Groups	Length (cm) (Mean ± SE) (Range)	Width (cm) (Mean ± SE) (Range)	Height (cm) (Mean ± SE) (Range)	Length (cm) (Mean ± SE) (Range)	Width (cm) (Mean ± SE) (Range)	Height (cm) (Mean ± SE) (Range)
G I (n=4)	4.2 ± 0.5^{a} 3.5-4.7	2.0 ± 0.4^{a} 1,4-2,4	2.6 ± 0.3^{a} 2.5-2.9	4.2 ± 0.2^{a} 4.0-4.5	1.7 ± 0.3^{a} 1.2-1.9	2.4 ± 0.3^{a} 2.0-2.6
GII (n=7)	5.6 ± 1.3^{ab} 3.5-7.2	2.8 ± 0.9^{b} 1,6-3,8	3.3 ± 0.8^{ab} 1.9-4.0	4.9 ± 0.9^{a} 36-6.0	2.4 ± 0.7^{ab} 1.7-3.9	2.9 ± 0.7^{a} 1.9-3.9
GIII (n=14)	6.1 ± 1.1^{bc} 4.0-8.0	3.1 ± 0.6^{b} 1.8-3.7	3.7 ± 0.6^{bc} 2.4-4.5	5.6 ± 1.2^{ab} 3.5-7.6	2.9 ± 0.6^{bc} 1.5-3.5	3.4 ± 0.7^{ab} 1.7-4.4
GIV (n=9)	7.5 ± 0.5^{c} 7.0-8.7	4.0 ± 0.3^{c} 3.6-4.7	4.4 ± 0.4^{c} 4.0-5.4	6.7 ± 0.9^{b} 4.9-7.6	3.4 ± 0.6^{c} 2.4-4.1	4.0 ± 0.7^{b} 2.7-4.8

a,b,c different superscripts in column indicate statistical difference ($P<0.05$)

(P=0.001). With the exception of one horse that had high testicular volume and weight, but no spermatozoon, all animals presented epididymal sperm when testicular volume and weight were over 16 cm^3 and 23 g, respectively.

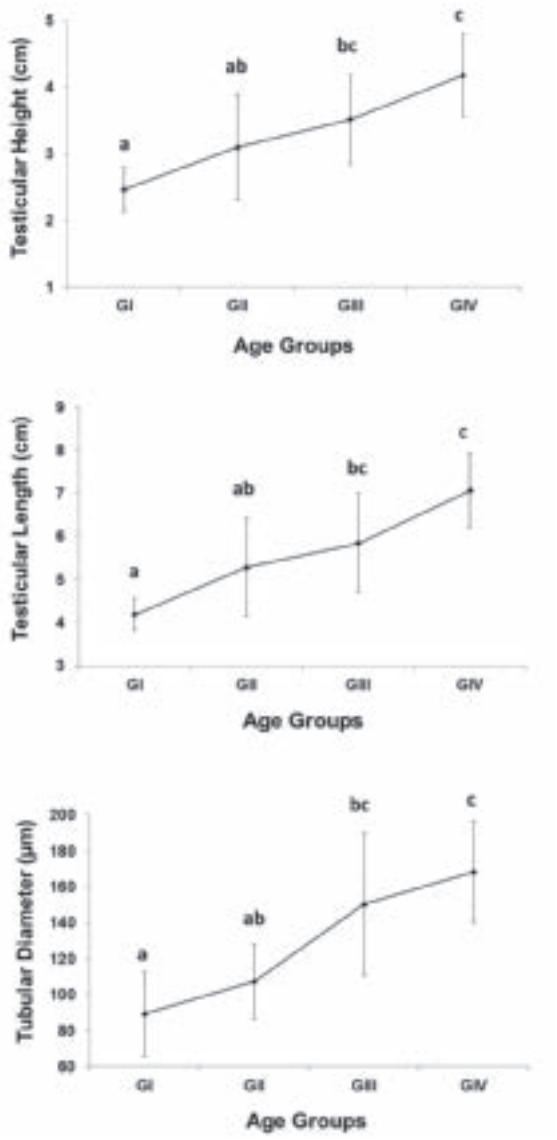

Fig. 2 Diameter of seminiferous tubules, testicular height and length in horses. [a,b,c] different superscripts indicate statistical difference (P<0.05)
Durchmesser der Samenkanälchen, der Hodengröße und Hodenlänge bei Pferden. [a,b,c] Verschiedene obere Buchstaben zeigen statistischen Unterschied an (P<0.05)

In GI, the tubular diameter reached approximately 89.13±23.62 µm, and increased to 168.24±28.08 µm in GIV (P=0.0044). In GII and GIII, the mean tubular diameter was 107.24±20.92 µm and 150.20±39.56 µm, respectively. The diameter of the seminiferous tubules showed the same pattern of growth of testicular height and length (Fig. 2).

Discussion

Testes measurements were related to the appearance of more advanced germ cell types, suggesting that gonad volume and weight were mainly determined by percentage of these cells. As previously shown by *Moura* et al. (2011), as animal age and proliferation of germ cells increases, the number of these cells starts to affect testis weight. In GIV animals seminiferous tubules with large quantities of mature spermatids were found, but not in GI. These findings suggest that one year old horses were in pre-puberal gonadal development (*Johnson* et al., 1991). From seventeen months of age a significant numbers of mature spermatids were identified in the seminiferous tubules. Based on this evidence, these can be considered near puberty and according to *Johnson* (1995) the efficiency of spermatogenesis is related to the amount of germ cell degeneration, pubertal development, season of the year, and aging of humans and animals. In this study, with the exception of one horse, that had high testicular volume and weight, but no spermatozoa, epididymal sperm were present, when testicular volume and weight exceeded 16 cm^3 and 23 g, respectively. No research was found that relates testicular size and weight to the beginning of the sperm production and puberty.

As noted in previous reports, testicular weight and dimension vary significantly in sexually immature stallions (*Johnson* and *Thompson* 1983, *Pickett* et al. 1989, *Naden* et al. 1990, *Melo* et al. 1998). Testicular volume, estimated by measurements, is correlated to sperm production (*Johnson* et al. 1994, *Love* et al. 1991, *Quartuccio* et al. 2011) and small testicular size can be associated with reduced sperm production (*Figueiró* 2010). There was no difference (P>0.05) between the left and right testis for any weight or size measurement in horses, which is in accordance with *Parlevliet* et al. (1994). However, *Nishikawa* (1959), *Gebauer* et al. (1974), *Paccamonti* et al. (1999) and *Kavak* et al. (2003), report tendencies of the left testicle to be larger than the right. The reason for the left–right variation of testis size is not known, but *Nishikawa* (1959) suggests that the left testis developed earlier and grew more rapidly from one month to 4-5 years in 80% of the horses. In this experiment the mean testicular

Table 2 Development of seminiferous epithelium of horses as related to the most advanced germ cell type (mean ± standard error)
Entwicklung des Keimepithels bei Pferden bezogen auf den höchstentwickelten Keimzelltyp (Durscnitt ± Standardfehler)

	Percentage of cross sections of seminiferous tubules with the most developed germ cell type (mean ± standard error)					
Age Groups	Tubules without germ cells	Tubules with Spermatogonia	Tubules with Spermatocytes	Tubules with Round spermatids	Tubules with Mature spermatids	Tubules with Spermatozoa
GI (n=4)	17.5±4.7^a	57.5±8.9^a	22.5±11.2^a	2.5±2.0^b	0	0
GII (n=7)	5.4±3.6^{ab}	28.9±6.8^{ab}	40.7±4.3^a	13.9±4.8^{ab}	10.4±5.5^b	0.4±0.4^b
GIII (n=14)	5.2±5.5^{ab}	13.6±8.9^b	27.7±6.9^a	21.4±5.7^a	25.0±7.1^{ab}	5.5±4.0^b
GIV (n=9)	0.8±1.0^b	8.9±6.1^b	16.4±10^a	12.8±3.4^{ab}	36.4±8.2^a	25.3±6.2^a

a,b different superscripts in column indicate statistical difference (P<0.05)

measurements (length, width and height) of horses in GI were similar to the values found in Pantaneiro colts aged between 12 and 15 months (*Melo* et al. 1998). In relation to testicular weight, the colts in GI had heavier testis than the Pantaneiro horse. All testicular measurements and testicular weight of horses between 19 and 34 months can be compared to the results obtained in Pantaneiro stallions between 26 and 28 months, according to *Melo* et al.(1998) and as described by *Naden* et al. (1990) for Quarter Horse stallions at two years of age.

Testosterone concentrations showed no significant differences between the age groups. The results found in this study disagree from those described by *Naden* et al. (1990) and *Melo* et al. (1998) who described that serum testosterone levels increased with age. According to *Amann* (2011) three to eight episodic bursts of testosterone production occur each day in most horses. Consequently, if a single blood sample is taken as in this experiment, an unusually high value may be obtained. Furthermore the technique for measurement of testosterone used in this study was the chemiluminescence method, different from the assays used by most authors (*Naden* et al. 1990, *Johnson* et al. 1991, *Stewart* and *Roser* 1998, *Brown-Douglas* et al. 2005). The differences in testosterone concentration found in this experiment compared to other studies may be explained by these facts.

According to *Wesson* and *Ginther* (1981) the onset of puberty can be determined based on the presence of spermatozoa in the epididymis. Using this criterion, all animals with 20 months of age or more had reached puberty in the present study. The first spermatozoa appeared in 16 months old colts. According to *Skinner* and *Bowen* (1968), puberty in pony stallions occurs between 11 and 15 months of age. The presence of spermatozoa in the ejaculate can be detected between 14 and 24 months in Quarter Horse colts (*Naden* et al. 1990). In colts aged 12-13 months (summer born) and animals aged between 15 and 16 months (spring born), all spring-born colts and only one of four summer-born colts, had reached puberty at this age (*Wesson* and *Ginther* 1981).

The diameter of seminiferous tubules in horses increased with age. In GII and GIII the mean of tubular diameter was 107.24±20.92 μm and 150.20±39.56 μm, respectively. *Swierstra* et al. (1974) evaluated the diameters of the seminiferous tubules, comparing the eight stages of the seminiferous epithelium in two groups of mature stallions. The mean tubular diameters for stages varied between 156±3 μm and 161±2 μm, suggesting a similarity between values obtained in adult stallions (3-10 years of age) and those found in animals of groups GIII and GIV in this study. However, *Figueiró* (2010) reported an average for seminiferous tubule diameter of 205 μm in mature stallions. This may indicate that the testicular development was ongoing in the animals of the present study.

In conclusion animals with 20 months of age or more had reached puberty in the present study and the first spermatozoa appeared in 16 months old colts. With the exception of one horse that had high testicular volume and weight, but no spermatozoon, epididymal sperm were presented, when testicular volume and weight were over 16 cm^3 and 23 g, respectively.

References

Abercrombie M. (1946) Estimation of nuclear population from microtome sections. Anatom. rec. J. 94, 239-247

Aguiar G. V., Araújo A. A. and *Moura A. A. A.* (2006) Desenvolvimento testicular, espermatogênese e concentrações hormonais em touros Angus. Revista Brasil. Zoot. 35, Supplement 4, 1629-1638

Amann R. P. (2011) Physiology and Endocrinology. In: McKinnon A. O., Squires E. L., Vaala W. E., Varner D. D. Equine Reproduction. 2. Ed. Wiley-Blackwell v. 1, 881-908

Brown-Douglas C. G., Parkinson T. J., Firth E. C. and *Fennessy P. F.* (2005) Bodyweights and growth rates of spring- and autumn-born Thoroughbred horses raised on pasture. New Zeal. Vet. J. 53, 326-331

Bruemmer J. E. (2006) Collection and freezing of epididymal stallion sperm. Vete. Clin. Equine 22, 677-682

Dowsett K. F and *Pattie W. A.* (1982) Characteristics and fertility of stallion semen. Reprod. Fertil., Suppl. 32, 1-8

Dowsett K. F and *Pattie W. A.* (1987) Variation in characteristics of stallion semen caused by breed, age, season of year and service frequency. Reprod. Fertil., Suppl. 35, 645-647

Figueiró G. M. (2010) Análise morfofuncional da espermatogênese do cavalo da raça Crioula. Tese de Doutorado. Santa Maria – RS, Universidade Federal de Santa Maria.

Gebauer M. R., Pickett B. W. and *Swierstra E. E.* (1974) Reproductive physiology of the stallion. II. Daily production and output of sperm. Animal Sci. 39, 732-736

Johnson L., Blanchard T. L., Varner D. D. and *Scrutchfield W. L.* (1997) Factors affecting spermatogenesis in the stallion. Theriogenology 48, 1199-1216

Johnson L. and *Thompson D. L. Jr.* (1983) Age-related and seasonal variation in the Sertoli cell population, daily sperm production and serum concentrations of follicle-stimulating hormone, luteinizing hormone and testosterone in stallions. Biol. Reprod. 29 777-789

Johnson L. and *Thompson D. L. Jr.* (1987) Effect of seasonal changes in Leydig cell number on the volume of smooth endoplasmic reticulum in Leydig cells and intratesticular testosterone content in stallions. Reprod. Fertil. 81, 227-232

Johnson L., Varner D. D. and *Thompson D. L.* (1991) Effect of age and season on the establishment of spermatogenesis in the horse. Reprod. Fertil., Supplement 44, 87-97

Johnson L., Carter G. K., Varner D. D., Taylor T. S., Blanchard T. L. and *Rembert M. S.* (1994) The relationship of daily sperm production with number of Sertoli cells and testicular size in adult horses: role of primitive spermatogonia. Reprod. Fertil. 100, 315-321

Johnson L. (1995) Efficiency of spermatogenesis. Microsc. Res. Tech. 32, 385-422

Kavak A., Lundeheim N., Aidnik M. and *Einarsson S.* (2003) Testicular measurements and Daily Sperm Output of Tori and Estonian Breed Stallions. Reprod. Dom. Anim. 38, 167-169

Love C. C., Garcia M. C., Riera F. R. and *Kenney R. M.* (1991) Evaluation of measures taken by ultrasonography and caliper to estimate testicular volume and predict daily sperm output in the stallion. Reprod. Fertil., Supplement 44, 99-105

Melo M. I. V., Sereno J. R. B., Henry M. and *Cassali G. D.* (1998) Peripuberal sexual development of Pantaneiro stallions. Theriogenology 50, 727-737

Moura A. A. and *Erickson B. H.* (2011) Testicular development, histology, and hormone profiles in three yearling Angus Bulls with spermatogenic arrest. Theriogenology 55, 1469-1488

Moura A. A., Souza C. E. A. and *Erickson B. H.* (2011) Early prepubertal testis criteria, seminiferous epithelium and hormone concentrations as related to testicular development in beef bulls. Anim. Reprod. Sci. 124, 39-47

Naden J., Amann R. P. and *Squires E. L.* (1990) Testicular growth, hormone concentrations, seminal characteristics and sexual behaviour in stallions. Reprod. Fertil. 88, 167-176

Neves E. S., Chiarini-Garcia H. and *França L. R.* (2002) Comparative Testis Morphometry and Seminiferous Epithelium Cycle Length in Donkeys and Mules. Biol. Reprod. 67, 247-255

Neves A. P., Trein C. R., Möller G., Mattos R. C. and *Klug E.* (2005) Reproductive parameters of Mini-Shetland stallions in north Germany. Anim. Reprod. Sci. 89, 267-270.

Nishikawa Y. and *Horie T.* (1955) Studies on the development of the testes and epididymides of the horse. I. Studies on the development of the testes of the horse, with special reference to singularity and the age of sexual maturity. Bull. natn. Inst. agrie Sci. 10, 299

Paccamonti D. L., Buiten A. V., Parlevliet J. M. and *Colenbrander B.* (1999) Reproductive parameters of Miniature stallions. Theriogenology 51, 1343-1349

Parlevliet J. M., Kemp B. and *Colenbrande B.* (1994) Reproductive characteristics and semen quality in maiden Dutch Warmblood stallions. Reprod. Ferti. 101, 183-187

Pickett B. W., Voss J. L., Bowen R. A., Squires E. L. and *McKinnon A. O.* (1987) Seminal characteristics and total scrotal width (T.S.W.) of normal and abnormal stallions. Proceedings of the 33rd Annual American Association of Equine Practitioners Convention 487-518

Pickett,B. W., Amann R. P., McKinnon A. O., Squires E. X. and *Voss J. L.* (1989) Management of the stallion for maximum reproductive efficiency. II. Animal Reproduction Laboratory Bulleting No. 05. Fort Collins, Colorado State University

Quartuccio M., Marino G., Zanghì A., Garufi G. and *Cristarella S.* (2011) Testicular Volume and Daily Sperm Output in Ragusano Donkeys. Equine Vet. Sci. 31, 143-146

Remiezowicz A., Mattos R.C., Neves A.P., Müller K., Ellenberger C. and *Schoon H. A.* (2008) Small but dynamite: Brazilian pony stallions have more testicular Leydig cells than German warm blood stallions. Anim. Reprod. Sci. 107, 345-346

Skinner J. D. and *Bowen J.* (1968) Puberty in the Welsh Stallion. Reprod. Fertil. 16, 133-135

Squires E. L. and *Pickett B W.* (2011) Factors affecting sperm production and output. In: McKinnon A. O., Squires E. L., Vaala W. E and Varner D. D. Equine Reproduction. 2. Ed. Wiley-Blackwell, v.1, 1344-1361

Stewart B. L. and *Roser J. F.* (1998) Effects of age, season, and fertility status on plasma and intratesticular immunoreactive (IR) inhibin concentrations in stallions. Domestic Animal Endocrinology 15, 129-139

Swierstra E. E., Gebauer M. R. and *Pickett B. W.* (1974) Reproductive physiology of the Stallion I. Spermatogenesis and testis composition. Reprod. Fertil. 40, 113-123

Voss J. L, Pickett B. W. and *Loomis P. R.* (1982) The relationship between semen characteristics and fertility in Thoroughbred stallions. Reprod. Fertil., Supplement 32, 635-636

Wesson J. A. and *Ginther O. J.* (1981) Puberty in the male pony: plasma gonadotrophin concentrations and the effects of castration. Animal Reprod. Sci. 4, 165-175

Dr Joana Weber Gregory
REPROLAB, Veterinary Faculty
Federal University of Rio Grande do Sul
Estrada Dois 399 - Parque São Paulo-Lami
91787-762 Porto Alegre/RS
Brasil
joanagregory@yahoo.com.br