A study of serum insulin-like growth factor type 1 (IGF-1) concentrations in resting untrained Andalusian horses: influence of age and gender

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ABSTRACT: Growth rate, tissue repair and reproductive functions are mediated by the somatotrophic axis, the growth hormone (GH) being one of its main components. GH is released in a pulsatile manner and a single measurement does not provide accurate information on the activity of the somatotrophic axis. The actions of GH on tissues are mediated by insulin-like growth factor type 1 (IGF-1), mainly released by the liver, and thus, the measurement of IGF-1 could be considered a good indicator of the activity of GH and the somatotrophic axis. Serum IGF-1 concentrations are relatively stable due to its long biological half-life without obvious diurnal rhythm. Additionally, many diseases significantly alter circulating IGF-1 concentrations, leading to potential diagnostic and prognostic uses in veterinary medicine. However, serum IGF-1 concentrations are affected by many factors, such as breed, age and sex. The present study analyzes the influence of these factors on serum IGF-1 concentrations in a population of 255 Andalusian horses (141 females and 114 males), divided into age groups: 1–2, 2–3, 3–4, 4–5, 5–6 and 6–12 months and 1–2, 2–4, 4–6, 6–10 and 10–14 years. The animals belonged to six different farms located in the same geographic location and were subjected to similar feeding and management protocols. Two measurements of body size were made: height at the withers (HW) and diameter of the thorax (DTx). Blood samples were taken always in the morning, in the month of July and serum IGF-1 concentrations were measured with a sandwich ELISA after dissociation of IGF-1 from its binding proteins. It was found that age and sex significantly influenced serum IGF-1 concentrations, whereas the effects of the farm and the time of blood withdrawal were not significant. Mean serum concentrations for both males and females respectively were: 246.3 and 231.0 (1–2 months), 201.9 and 194.7 (2–3 months), 174.2 and 170.4 (3–4 months), 161.7 and 155.4 (4–5 months), 166.1 and 136.9 (5–6 months), 127.2–114.5 (6–12 months), 103.3 and 89.01 (1–2 years), 104.3 and 73.41 (2–4 years), 105.4 and 64.40 (4–6 years), 53.29 and 68.27 (6–10 years) and 59.56 and 65.53 ng/ml (10–14 years). A progressive decrease in serum IGF-1 concentrations with increased age was found for both sexes. Males aged between five and 12 months and between two and six years had significantly higher serum IGF-1 concentrations than females of the same age. Coefficients of correlation between the indicators of body size (HW and DTx) and IGF-1 were –0.800 and –0.690 for the whole population of Andalusian horses, –0.860 and –0.750 for the males and –0.740 and –630 for the females. It is concluded that serum IGF-1 concentrations in Andalusian horses are reduced with ageing, male horses of determined age groups had higher IGF-1 than the females and there are negative correlations between body size and IGF-1 concentrations. The knowledge of the normal serum IGF-1 concentrations will help us to understand the role of the somatotrophic axis in several diseases and physiological situations and will provide information for further research on this equine breed.

Keywords: age; growth hormone; horse; insulin-like growth factor; sex

Growth rate is regulated mainly by the somatotrophic axis, a main component in the chemical communication network that links metabolism to nutrient intake (LeRoith et al., 2001; Champion et al., 2002; Yakar et al., 2002; Staniar et al., 2007). The somatotrophic axis is integrated by thyroid

hormones, glucocorticoids, leptin, and steroid hormones, although their main components are insulin and growth hormone (GH) or somatotropin (ST). GH is produced by the anterior pituitary gland and is secreted in a pulsatile nature in horses (Thompson et al., 1992; Stewart et al., 1993; DePew et al., 1994), as well as in other species (Plouzek and Trenkle, 1991; Breier and Sauerwein, 1995). The actions of GH on tissues are partly mediated by insulin-like growth factor 1 (IGF-1), which is secreted mainly from the liver but also from other non-hepatic tissues to act in an endocrine-autocrine-paracrine fashion (Ovesen et al., 1996). Plasma concentrations of IGF-1 in the bloodstream of man and domestic animals are relatively stable because of its long biological half-life, with no obvious diurnal rhythm (Gluckman et al., 1987; Breier and Sauerwein, 1995). As a consequence, the measurement of IGF-1 is very useful in determining the level of activity of the somatotrophic axis as GH measurements provide very little information due to its episodic release.

Serum IGF-1 concentrations in horses depend on many factors, such as breed (Tremblay et al., 1993; Ozawa et al., 1995), age (Malinowski et al., 1996; Champion et al., 2002; Fortier et al., 2005; Noble et al., 2007; Munoz et al., 2010a), sex (Ozawa et al., 1995), nutritional status, particularly dietary protein supply and feed deprivation and refeeding (Sticker et al., 1995; Christensen et al., 1997; Ropp et al., 2003), exercise, training and fitness level (Jackson et al., 2003; Noble et al., 2007).

A positive relationship between body size and circulating IGF-1 concentrations has been reported in dogs and pigs. Thus, the German shepherd breed has higher IGF-1 concentrations than smaller breeds (Eigenmann et al., 1984) and similarly, large (normal) poodles have larger plasma IGF-1 concentrations than toy poodles (Eigenmann et al., 1984). In the same way, macro pigs showed higher IGF-1 concentrations than miniature or micro pigs (Buonomo et al., 1987). Ozawa et al. (1995) compared IGF-1 concentrations in heavy horses (Percheron and Breton breeds), light horses (Thoroughbreds) and ponies (Shetland and Falabella). Despite the positive correlations between body weight and plasma IGF-1 concentrations, the means in heavy horses did not exceed that of light horses or ponies with smaller body weights (Ozawa et al., 1995). On the other hand, Tremblay et al. (1993) found that Standardbred horses had higher circulating IGF-1 concentrations than Thoroughbreds. Serum IGF-1 concentrations have been reported in horses of different breeds, such as Thoroughbreds (Ozawa et al., 1995; Jackson et al., 2003; Fortier et al., 2005; Noble et al., 2007), Standardbred trotters (Malinowski et al., 1996; Champion et al., 2002), Belgian-cross and Quarter Horse-draft cross (Cymbaluk and Laarveld, 1996) and Quarter Horses (Ropp et al., 2003). To the authors' knowledge, the studies concerning comparative interbreeds are more limited (Tremblay et al., 1993; Ozawa et al., 1995; Cymbaluk and Laarveld, 1996).

The influence of age on serum IGF-1 concentrations has been analyzed by several authors in different equine breeds. In Standardbreds, Malinowski et al. (1996) found that IGF-1 concentrations increased continuously from birth to Day 14, then remained constant until nine months of age and significantly decreased in older mares (22 years). Similarly, Champion et al. (2002) observed that the highest IGF-1 concentrations occurred in one-year-old foals, with a steady decrease to three-year-old foals. These authors did not find significant differences from three to eight years of age. In Thoroughbreds, Fortier et al. (2005) found the highest IGF-1 concentrations at Day 9 of age, which steadily declined to Day 224 (approximately 7.5 months). After Day 224, serum IGF-1 concentrations increased to a level comparable to neonatal concentrations, and then again decreased continuously to Day 450 (approximately 15 months), after which time they remained relatively steady to Day 715 (approximately 31.8 months). The higher IGF-1 concentrations during the most rapid growth period of foals are consistent with reports from other species (Breier et al., 1994).

Sex is another factor to be taken into account when considering circulating IGF-1 concentrations in horses. Ozawa et al. (1995) found higher plasma IGF-1 concentrations in male than in female horses of different breeds. Similarly, Champion et al. (2002) and Noble et al. (2007) reported higher IGF-1 concentrations in intact males compared with mares and geldings in Standardbred and Thoroughbred horses, respectively.

The circulating concentrations of IGF-1 vary in different diseases and therefore, could serve as diagnostic and prognostic tools. A review of the changes of this parameter in liver, heart, endocrine, locomotor diseases and in neoplasias in veterinary medicine has been recently published (Munoz et al., 2010b). In summary, it has been confirmed that IGF-1 concentrations are reduced

in dogs with liver diseases (Neumann et al., 2007), congenital portosystemic shunts (Maxwell et al., 2000) and mitral regurgitation (Pedersen et al., 2005). In horses, decreased circulating IGF-1 concentrations have been found in osteochondrosis (Sloet van Oldruitenborgh-Oosterbaan et al., 1999), juvenile digital osteoarthropathy (Lejeune et al., 2007) and in several joint diseases (Verwilghen et al., 2009). In contrast, some disorders associated with insulin resistance, such as pars intermedia pituitary dysfunction, equine metabolic syndrome, pasture-associated laminitis and hyperlipemia might be related to high serum IGF-1 concentrations (Munoz et al., 2010b).

The research presented here aims to provide a profile for serum IGF-1 concentrations in a normal and healthy population of Andalusian horses and to ascertain whether age, sex and body size have an effect. To the authors' knowledge, the reference range for IGF-1 has not been previously reported for this equine breed. The establishment of the normal serum IGF-1 concentrations will enable a greater understanding of the role of the somatotrophic axis in different diseases and physiological situations, providing information for further studies.

MATERIAL AND METHODS

Animals

The procedures followed in this research were approved by the animal care and ethics committee of the University of Cordoba, Spain. Two hundred and fifty five Andalusian horses were included in the study, of both genders (141 females and 114 males), and with ages ranging between one month and 14 years. As this research evaluated the effect of age on serum IGF-1 concentrations, the horses were divided into age groups, as specified in Table 1. The animals belonged to six different farms situated in the same geographic location.

All the animals were ascertained as healthy on the basis of physical examination, laboratory analysis (haematology and biochemistry, including fibrinogen determination) and no obvious orthopaedic diseases were found. Additionally, horses older than four years were radiologically tested for osteochondrosis/osteochondrosis dissecans and only those animals found to be free of this disorder were studied. All the horses were subjected to the same deworming and vaccinations protocols (influenza, tetanus and rhinopneumonitis). The animals were kept in paddocks, separated by age and the stallions were in individual boxes. Feeding consisted of oats and alfalfa hay, with free access to water. The mares and the foals older than six months were at pasture. They were not in active training at the moment of the research.

Table 1. Distribution by age and sex of the 255 Andalusian horses included in the present research

Age groups (months)	Females	Males	Total
1–2	8	8	16
2–3	10	8	18
3–4	10	10	20
4–5	6	10	16
5–6	6	10	16
6–12	14	10	24
12–24 (1–2 years)	11	12	23
25–48 (2–4 years)	19	14	33
49–72 (4–6 years)	17	12	29
73–120 (6–10 years)	25	10	35
121–144 (10–14 years)	15	10	25

Physical measurements

The height at the withers (HW) was measured with a standard measuring stick (Hauptner®, Herberholz, Dietlikon-Zurich, Switzerland) from the highest point of the withers after ensuring a good standing position. The diameter of the thorax (DTx) was measured with a standard tape behind the elbow at the time of respiratory expiration.

Blood sample withdrawal and serum IGF-1 measurements

Jugular venous blood samples were extracted at basal conditions, before feeding, when possible. In suckling foals, the time of blood sampling in relation to nursing was not controlled, but in animals older than six months, the time between feeding concentrates and blood samples was recorded. All the samples were drawn in the same season of the year (in the month of July) and between 09:00 and 14:00 hours.

After withdrawal, blood samples were transferred into tubes with EDTA and in tubes with activators of coagulation. EDTA-blood was used to perform a haematological study in order to assess health status. Serum was harvested from the tubes with activators of coagulation to establish a biochemical profile and to measure serum IGF-1 concentrations. All the samples were kept on ice after withdrawal until they were transported to the laboratory. Serum for IGF-1 measurements were frozen for further analysis, which was carried out within the first two months after extraction.

Serum IGF-1 concentrations were measured with a sandwich ELISA (DRG® International Inc, IGF-1 equine, EIA-3982, USA). IGF-1 circulating in the bloodstream binds to IGFBP (insulin-like growth factor binding proteins) and therefore, an extraction of IGFBP was made. This extraction was carried out using a monoclonal anti-IGF-1 antibody. This method was very specific for equine IGF-1, with a cross-reactivity of lower than 0.01% for IGF-2 and lower than 0.1% for insulin and GH. The minimum concentration of IGF-1 detected by this method was 4.9 ng/ml. Intra- and inter-assay coefficients of variation were 8.033% and 12.77%, respectively. The percentage of recovery of IGF-1 from known concentrations ranged between 101.3% and 107.2%. The assay has been validated for horses (information provided by the manufacturer).

Statistical procedures

Tests for normal distribution of serum IGF-1 concentrations in the whole population of Andalusian horses were performed using the Shapiro-Wilk univariate test. Data concerning greatest and lowest concentrations, 99th percentile, Shapiro-Wilk univariate test, skewness and kurtosis were calculated. Positive values for skewness indicated that values above the mean are more spread out than values below the mean. Positive values for kurtosis indicated more extreme values than a normal population.

The effects of age, sex, time of blood withdrawal and farm on IGF-1 concentrations were determined using an ANOVA, with main effects and interactions sought for each individual variable. When statistical significance was reached, a Tukey test was used to assess between which groups there were significant differences. The correlations between body size (HW and DTx) and serum IGF-1 concentrations were investigated with a linear correlation test (Pearson test). Statistical significance was fixed at $P < 0.05$. The program Statistica® for windows was used.

RESULTS

Results are presented as means ± SD. Data on body size indicators for each age group and sex are presented in Table 2. Tests for a normal dis-

Table 2. Indicators of body size (HW, height at the withers and DTx, diameter of the thorax) in 255 Andalusian horses separated by age and gender

Age groups (months)	Females		Males	
	HW (cm)	DTx (cm)	HW (cm)	DTx (cm)
1–2	110.9 ± 3.45	108.4 ± 4.50	118.8 ± 3.44*	112.9 ± 3.64
2–3	121.8 ± 4.35	123.0 ± 3.40	123.6 ± 2.97	131.2 ± 2.56*
3–4	122.7 ± 3.44	119.1 ± 4.60	124.6 ± 4.69	126.2 ± 4.37
4–5	124.3 ± 2.96	125.6 ± 5.60	125.3 ± 5.32	127.0 ± 4.30
5–6	128.5 ± 3.10	118.5 ± 3.55	133.2 ± 5.33	140.3 ± 3.55*
6–12	131.2 ± 10.2	140.3 ± 12.3	130.2 ± 6.50	141.2 ± 9.50
12–24 (1–2 years)	141.5 ± 5.40	173.8 ± 5.60	154.2 ± 4.30*	155.0 ± 6.50*
25–48 (2–4 years)	155.3 ± 5.43	177.8 ± 6.55	157.5 ± 4.33	167.1 ± 7.60
49–72 (4–6 years)	152.8 ± 6.43	181.3 ± 6.49	154.7 ± 2.69	173.3 ± 4.50*
73–120 (6–10 years)	155.8 ± 5.90	176.0 ± 5.60	157.3 ± 4.97	168.0 ± 3.49*
121–144 (10–14 years)	155.5 ± 6.40	169.4 ± 7.69	159.8 ± 5.40	168.5 ± 4.21

*significant differences between males and females for each age groups; $P < 0.05$)

Table 3. Serum IGF-1 concentrations in a population of 255 Andalusian horses, including statistical tests for normality

Group	Age (months)#	IGF-1 (ng/ml)#	Concentration (ng/ml)		99th percentile (ng/ml)	Shapiro-Wilk univariate test	*P*-value	Skewness	Kurtosis
			greatest	lowest					
Whole population (*n* = 255)	52.58 ± 51.10	116.7 ± 62.49	273.5	30.80	270.2	0.910	< 0.001	0.725	0.511
Females (*n* = 141)	61.51 ± 52.28	109.0 ± 63.35	273.5	30.82	173.5	0.864	< 0.001	0.945	0.183
Males (*n* = 114)	60.24 ± 45.38	132.7 ± 58.17	270.2	36.07	270.2	0.960	< 0.001	0.892	0.614

#mean ± SD

tribution among the entire population and within groups of each sex are presented in Table 3. The Shapiro-Wilk statistic test showed that the distribution significantly deviated from a normal one, with a positive test for skewness. The positive kurtosis indicated that the distribution plot had heavy tails, which confirmed that there were more values at the extremes of the distribution.

The results of the ANOVA test and the interactions between the different factors that could have influenced the serum IGF-1 concentrations in the Andalusian horses are presented in Table 4. Significant effects of age, sex and the interactions between them were found. However, the effects of the farm and the time of blood withdrawal were not significant.

The serum IGF-1 concentrations in Andalusian horses of different age and sex are depicted in Figure 1. A significant effect of age was found in both females and males. Similarly, there were some significant differences associated with gender. Males aged between five months and 12 months and between two and six years had higher serum IGF-1 concentrations than females of the same age (Figure 1).

Table 4. Results of the multivariate analysis considering the different factors that could influence serum IGF-1 concentrations in Andalusian horses, as well as their interactions

Effects and interactions	*P*-value
Age	< 0.001
Sex	< 0.05
Time of blood withdrawal	n.s.
Farm	n.s.
Age × sex	< 0.05
Age × time of blood withdrawal	n.s.
Age × farm	n.s.
Sex × time of blood withdrawal	n.s.
Sex × farm	n.s.
Age × sex × time of blood withdrawal × farm	n.s.

n.s. = non-significant; $P < 0.05$

Significant negative correlations were found between the indicators of body size (HT and DTx) for the whole population and for both sexes. Correlation's coefficients between IGF-1 and HT and IGF-1 and DTx were –0.800 and –0.690, for the whole population, –0.860 and –0.750 for the males and –0.740 and –0.630 for the females respectively. These results are shown in Figure 2.

DISCUSSION

Growth rate, size and certain adaptations to environmental conditions are regulated by the somatotrophic axis, with its two main components, GH and its effector, IGF-1. Further, they act as regulatory factors in skeletal growth and the development of a healthy skeleton is an key concern when raising horses (Yakar et al., 2002). Additionally, some diseases, such as liver, heart, kidney and metabolic diseases, present with significantly modified serum IGF-1 concentrations and as a consequence, it can be used as a marker of prognosis (Maxwell et al., 2000; Pedersen et al., 2005; Neumann et al., 2007; Munoz et al., 2010b), as well as an indicator of administration of GH in equine athletes (Champion et al., 2002). However, there are many factors that might affect the circulating IGF-1 concentrations, such as age, gender, body size and breed. In the present study, we have measured serum IGF-1 concentrations in Andalusian horses of both sexes and

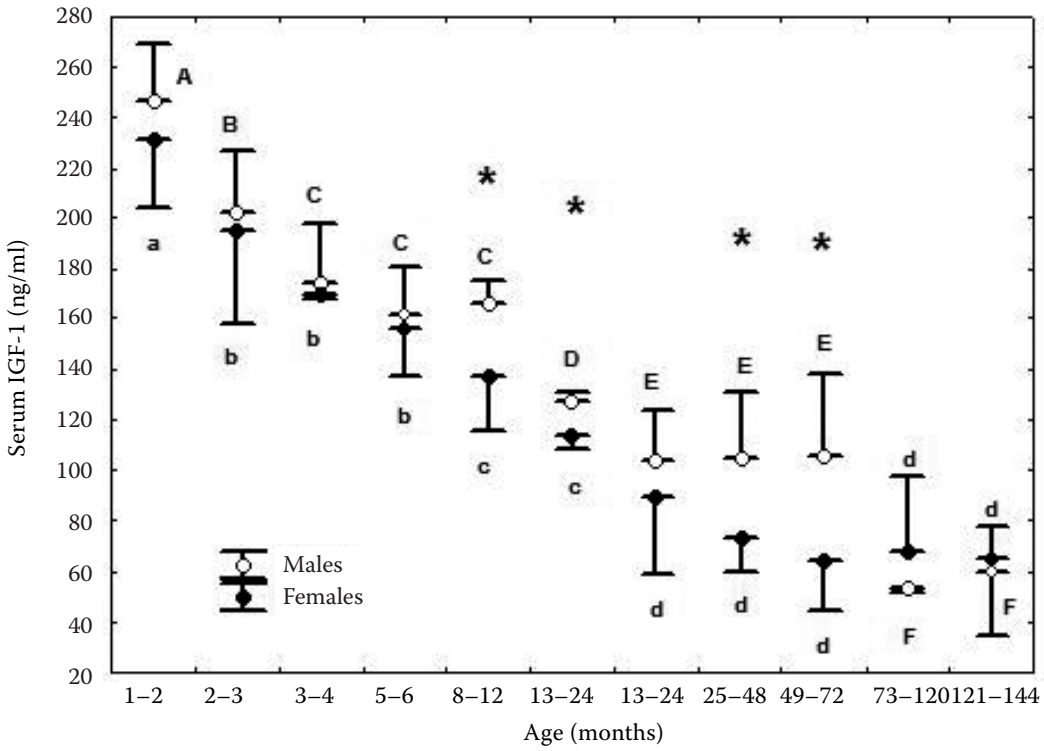

Figure 1. Mean (±SD) serum IGF-1 concentrations in male and female Andalusian horses aged between one month and 14 years. Different letters indicate significant differences between age groups for each sex (capital letters, A, B, C, D, E, F = Andalusian males; lower case letters, a, b, c d = Andalusian females)

*significant differences between males and females for each age group); $P < 0.05$

with ages ranging between one month and 14 years in order to establish reference values for these animals that can be used in further research studies. It was found that age was associated with a significant decrease in both males and females, although this decrease was slightly more intense in the males. Moreover, the Andalusian males aged between five and 12 months and between two and six years had higher serum IGF-1 than females of the same age. Finally, significant negative correlations were observed between IGF-1 and two indicators of body size, HT and DTx, and these correlations were higher for the Andalusian males.

A large percentage of circulating IGF-1 is bound to binding proteins, mainly to binding protein 3 (IGFBP-3), forming a high molecular weight tertiary complex (Blum, 1996). The dissociation of IGF-1 from its tightly associated binding protein is important for the measurement of total IGF-1 concentration. Due to the differing abilities of assays to dissociate these proteins, a comparison of the data obtained from Andalusian horses with other equine breeds is difficult and probably inaccurate. In any case, serum IGF-1 concentrations in the Andalusian horses were lower than those values reported for other breeds, such as Standardbreds (Malinowski et al., 1996; Christensen et al., 1997; Champion et al., 2002) and Thoroughbreds (Jackson et al., 2003; Noble et al., 2007). For instance, Malinowski et al. (1996) reported mean IGF-1 concentrations of between 597 and 530 ng/ml for Standardbred foals aged between one and nine months. These values were higher than those observed in Andalusian horses of the same age (serum IGF-1 concentrations between 240 and 120 ng/ml). We cannot say with confidence whether these results are down to the use of a different laboratorial method used to measure IGF-1 and/or from the different equine breeds. In all the studies previously mentioned, IGF-1 was separated from its binding proteins, although the procedure varied and therefore, these results should be interpreted with caution.

Design of the study

Although the literature concerning the chronology of the serum/plasma IGF-1 concentrations in

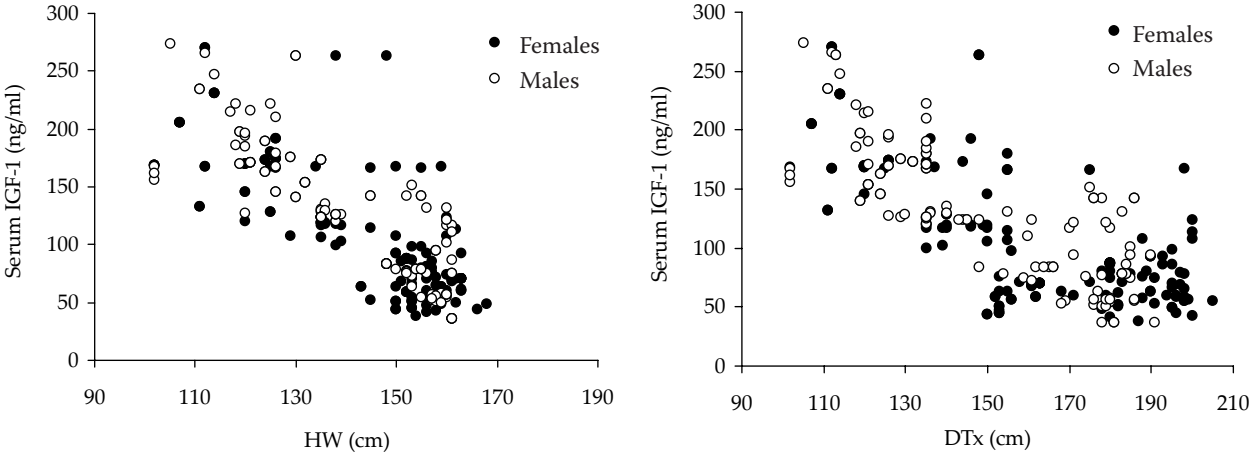

Figure 2. Scatter plot of the serum IGF-1 concentrations in males and females Andalusian horses in relation to height at the withers (HW) and diameter of the thorax (DTx); $P < 0.05$

horses during the post-natal period is limited, most studies have been performed comparing animals of different ages (Malinowski et al., 1996; Champion et al., 2002), with two longitudinal studies, one performed with a group of Quarter horse-draft crosses from one to 12 months of age and the other, with a group of growing Thoroughbreds from birth to 16 months of age (Cymbaluk and Laarveld, 1996; Staniar et al., 2007). It has been demonstrated that environmental conditions significantly influence pasture growth and composition and this fact would lead to different growth rates (Staniar et al., 2007). These last authors confirmed that there were clear seasonal patterns in both average daily gain and IGF-1 concentrations in growing Thoroughbred foals, with the highest values in June and May and the lowest in March in the North Hemispheric (Staniar et al., 2007). Similarly, positive associations between growth, IGF-1 concentrations, environmental temperature and other environmental conditions have been reported in cattle, deer and pigs (Ma et al., 1992; Sarko et al., 1994; Lincoln et al., 2001). The connection between the environmental conditions and the somatotrophic axis should regulate energy partitioning under changing environmental conditions. Because of these influences, we preferred to take the blood samples over the course of one week instead of carrying out a longitudinal study.

Another reason for taking the blood samples over a short period of time (two weeks) was to minimize the influence of the photoperiod. Longer daily photoperiod has been associated with increased plasma IGF-1 in dairy cows, and this increase resulted in increased milk yield (Dahl et al., 1997).

Moreover, it has been shown that differences in serum IGF-1 concentrations exist between geographic locations, and may be associated with nutritional factors or management (Champion et al., 2002). For that reason, and in order to avoid these influences that could bias our data, we studied animals belonging to several farms situated in the same geographic region, and subjected to similar feeding and management procedures.

Several previous studies have attempted to examine whether circulating IGF-1 concentrations follow a circadian rhythm in the horse, as presented for other hormones, such as cortisol (Popot et al., 2001; Jackson et al., 2003; Noble et al., 2007). Popot et al. (2001) observed no diurnal rhythm for IGF-1 but they collected blood samples from only three horses every hour from 05:00 to 21:00 h, disregarding the effect of night time. Subsequently, Jackson et al. (2003) used six horses, taking blood samples at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h, beginning in the morning. These authors identified an IGF-1 peak at 17:30 h, but the sampling regime could not be described as the zenith of a circadian cycle and additionally, they did not identify a nadir, which is pivotal to the determination of a circadian rhythm. Finally, Noble et al. (2007) identified several peaks of IGF-1, but these were small and occurred at irregular intervals. As a consequence, a clear circadian rhythm does not seem to govern serum IGF-1 concentrations in the horse. Therefore, it has been suggested that a single blood sample would be a reliable indication of IGF-1 status in the horse (Noble et al., 2007). In our research, we recorded blood sampling times,

and the multivariate analysis did not reveal significant differences in association with the time of blood extraction.

Circulating concentrations of IGF-1, as well as GH, are clearly dependent on nutritional status in different species, including the horse (Philips, 1986; Thissen et al., 1994; Sticker et al., 1995; Christensen et al., 1997; Ropp et al., 2003). Restricted nutrition and dietary protein supply seem to be an important limiting factor for maximal stimulation of circulating IGF-1 concentrations (Sticker et al., 1995; Christensen et al., 1997; Ropp et al., 2003). In fact, it has been demonstrated that food deprivation leads to reduced blood concentrations of IGF-1, which are reversed following refeeding in humans (Clemmons et al., 1981), mice (McKnight and Goddard, 1989), rats (Maes et al., 1983), pigs (Dauncey et al., 1990) and horses (Sticker et al., 1995; Christensen et al., 1997). According to the data provided by Christensen et al. (1997), a significant reduction in IGF-1 concentrations is apparent after 48 h of feed deprivation. In our study, the blood samples were taken during the morning. Therefore, some animals were in a fasted state, whereas others were already fed. Christensen et al. (1997) demonstrated that, although serum IGF-1 concentrations increased during the first 24 h after refeeding, this increase was not significant (increase from 198 to 209 ng/ml). As a consequence, we do not consider that the different periods of time between eating and blood sampling could have influenced our results. Additionally, we selected the farms of Andalusian horses that follow similar protocols of feeding. We did not score body condition in the studied horses, this being a point of limitation in our research, and although we measured some indicators of body size, such as HW and DTx, the animals were not weighed. Gentry et al. (2002) reported that circulating IGF-1 concentrations tended to be higher in mares with higher body condition score.

The effect of the exercise on serum IGF-1 concentrations in the horse is not clear, but is probably related to the intensity and duration. Jackson et al. (1998) reported that serum IGF-1 concentrations were decreased when horses were given intense treadmill exercise and increased after 40 min of walking on a mechanical horse walker. These results are in contrast with those presented by Champion et al. (2002), who found that circulating concentrations of IGF-1 remained relatively constant during moderate and high-intensity exercises. In our cases, all the samples were taken with the horses at rest. However, foals older than six months and the mares were in paddock whereas the stallions were in boxes. Therefore, the amount of daily exercise in pasture was different between genders. Unfortunately, this effect could not be controlled as this is the standard management procedure of the horses in Andalusian equine farms.

Little is known concerning the effect of training on serum IGF-1 concentrations in horses, even though it is possible that they are affected by fitness or training status. A long-term intense training regimen undertaken by swimmers resulted in increased concentrations of total and free IGF-1 (Koziris et al., 1999). In horses, however, Champion et al. (2002) failed to find any significant changes in serum IGF-1 concentrations in a large population of Standardbreds in training. Therefore, we selected for our study only those horses that were not in active training at the time of the experiment.

Influence of age on serum IGF-1 concentrations in Andalusian horses

As previously shown for other equine breeds, the highest serum IGF-1 concentrations were found in the youngest Andalusian horses. The relatively high serum IGF-1 in foals is consistent with the reports for other species (e.g. Luna et al., 1983 for human beings; Abribat et al., 1990 for cattle). However, the progressive reduction in serum IGF-1 concentrations detected in Andalusian horses from one month of age to 10–14 years is not in agreement with the chronological evolution presented for other equine breeds. Fortier et al. (2005) found high IGF-1 concentrations in Thoroughbred foals on Day 9 of age, with a decline to 7.5 months, increasing to a level comparable to neonatal concentrations and then again decreasing continuously to 15 months, after which time the concentrations remained relatively steady to 25 months of age. These authors found the peak concentrations at 8.5–10 months, whereas our peak concentrations were observed in the Andalusian foals aged 1–2 months. In Standardbreds, Malinowski et al. (1996) reported no significant decrease in serum IGF-1 concentrations during the first nine months of life. Therefore, it seems that although age leads to significant variations in circulating IGF-1 concentrations, the age at which these changes appear depend on the breed.

Influence of sex on serum IGF-1 concentrations in Andalusian horses

Serum IGF-1 concentrations are known to be greater in males of other species, including sheep (Gatford et al., 1997) and pigs (Owens et al., 1999) and this idea has been confirmed also in horses (Ozawa et al., 1995; Champion et al., 2002; Noble et al., 2007). Previous studies revealed that serum IGF-1 concentrations are higher in intact males (Champion et al., 2002; Noble et al., 2007; Staniar et al., 2007) and these results are in line with the data obtained in the present research into Andalusian horses. However, significant differences between both sexes were only found in foals aged 5–12 months and stallions aged 2–6 years. It is known that androgens increase the number of receptors for GH on the liver and therefore, greater release of IGF-1 to the bloodstream is expected (Clapper et al., 2000), even though it has been suggested that also the hepatic synthesis of IGF-1 is stimulated by both androgens and estrogens (McPherson et al., 2002). The period of time between two and four years of age is considered to constitute puberty in the Andalusian male and this fact could be one plausible explanation for the higher serum IGF-1 concentrations.

Relationships between serum IGF-1 concentrations and body size in Andalusian horses

A previous longitudinal study of Quarter Horse-Draft crosses from 1-12 months of age found that serum IGF-1 concentrations were correlated with body weight (Cymbaluk and Laarveld, 1996). This relationship between circulating IGF-1 and growth is conserved in various species. A positive correlation exists between IGF-1 and average daily gain in young pigs (Owens et al., 1999), cattle (Davis and Simmen, 2006) and horses (Staniar et al., 2007). In contrast, Malinowski et al. (1996) did not find a consistent relationship between adult blood IGF-1 and adult body size in several equine breeds. We found significant correlations between body size indicators (HW and DTx) and serum IGF-1, these correlations being more significant in the males than in the females. Our results seem to indicate that, within a determined breed, a correlation exists between body size and IGF-1, although the statistical significance of this correlation can be lost when comparing different breeds with different sizes and probably, different growth rates.

In summary, we have found that age exerts a significant influence on serum IGF-1 concentrations in Andalusian horses, whereas the effect of the sex is less evident. A progressive reduction was found in serum IGF-1 concentrations from one month of life to 14 years, both in females and males. Andalusian males aged between five and 12 months and between two and six years had higher IGF-1 concentrations than females of the same ages. Finally, significant negative correlations between IGF-1 and body size indicators were found, with this correlation being more significant for the males than for the females.

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