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Puberty and sexual maturity in Anglo-Nubian male goats raised in semi-intensive system

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ABSTRACT - The objective of this study was to characterize the sexual development in Anglo-Nubian male goats raised in semi-intensive system. Eight animals were monitored every fifteen days, from the 12th to the 44th week for age at penis detachment, live weight, scrotal circumference, seminal parameters (volume, aspect, concentration, wave motion, progressive individual motility, vigor and spermatic abnormalities) and serum testosterone levels. Serum testosterone levels were determined in blood samples collected at the 20th, 28th and 38th weeks of age. Penis detachment occurred at 102.9 ± 15.4 days of age. Live weight and scrotal circumference at 20th and 44th weeks of age ranged from 25.9 ± 3.5 to 44.7 ± 4.7 kg and from 21.2 ± 1.6 cm to 26.0 ± 1.5 cm, respectively. The ejaculate volume ranged from 0.38 ± 0.05 to 0.96 ± 0.04 mL, concentration ranged from 1.33 ± 0.64 to 3.54 ± 0.14.10^{9} mL^{-1} and the progressive individual motility varied from 48.0 ± 10.52% to 82.0 ± 3.74%, at the same age. The number of defective spermatozoa (major and minor) was 32.2 ± 5.8% vs. 8.80 ± 2.9% at the 20th and the 44th week of age, respectively. Serum testosterone level was 2.70 ± 1.40 ng.mL^{-1} at the 20th week, 8.50 ± 4.66 ng.mL^{-1} at the 28th week and 2.21 ± 2.28 ng.mL^{-1} at the 38th week of age. Age showed a correlation with live weight, scrotal circumference, and all the qualitative parameters of semen except for volume, with all other qualitative parameters of semen, and the correlation among sperm abnormalities and the other traits was negative. Serum testosterone levels and andrological parameters of Anglo-Nubian goats raised in semi-intensive system are closely related to age. In those animals, puberty is reached at the 20th week and sexual maturity is reached at the 38th week of age.

Key Words: andrologic parameters, semen, spermatic abnormalities, testosterone

Introduction

Goat production plays an important role in the socio-economic development in northeastern Brazil, especially concerning the subsistence and the supply of high biological value animal proteins to the low-income population (Carvalho Junior et al., 2009). On the other hand, the production indexes observed in most flocks without defined breed are still very low.

The introduction of exotic breeds that can potentially result in higher productivity has been used as a strategy for improving the efficiency of local goat flocks (Silva et al., 2006). Among the alternatives, the Anglo-Nubian breed has been one of the most genetic groups used in improvement programs involving goats in the semiarid region of Northeastern Brazil, because of its well adaptation to the climatic conditions of the region (Santos et al., 2005).

Introducing exotic breeds require a considerable number of male breeding stock and imposes the need of establishing selective criteria associated with the reproductive activity of the animals.

In order to understand the best management practices (Nishimura et al., 2000) and to establish the most appropriate selection criteria for young breeding stock, it is necessary to understand the reproductive physiology from the onset of puberty to the period of sexual maturity of the breeding goats. Furthermore, the earlier a breeding goat is selected and incorporated to a breeding program, the better it will be used and greater will be the return of the investment (Alves et al., 2006).

Puberty is characterized by the beginning of the reproductive activity and it has great importance for the breeding system in as much as it allows defining management practices such as the separation of lots defined by sex, time of castration, early selection of animals for procreation, permitting greater effectiveness in the improvement of the herd (Pacheco et al., 2009).

However, for reaching its full reproductive potential, the animal needs to reach its sexual maturity, which is a phase when the sexual instinct is shown, the mating capacity and seminal parameters consistent with the full reproduction (Pacheco et al., 2009).

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Therefore, the objective of this study was to characterize the sexual development of male Anglo-Nubian goats raised in semi-intensive systems.

Material and Methods

This study was conducted from February 2008 to October 2009, in the Laboratório de Reprodução de Caprinos e Ovinos at Universidade Estadual do Sudoeste da Bahia (14º53'S, 40°48'W; altitude of 874.8 m). According to Koppen classification, the climate in the region is the altitude tropical climate (Cwa), with an average annual temperature of 19.5ºC (15-25ºC) and an annual mean rainfall of 741 mm (Inmet, 2010).

It was collected 208 semen samples every fifteen days from eight Anglo-Nubian goats at 12 to 44 weeks of age. The animals were kept under a semi-intensive system (African Star Grass pasture - *Cynodon nlemfluensis* and 18% CP concentrate, 1% of live weight, water and mineral salt (*ad libitum*).

Semen collections were initiated after penis detachment and complete mating with ejaculation into an artificial vagina. Penis detachment - daily monitored from the 12th week of age - was admitted when there was complete release and exposure of the penis.

Puberty was reached when the animal showed a full sequence of sexual behavior, penis exposure, mating and ejaculation, and motile sperm in the ejaculate (Simplicio et al., 2000). Sexual maturity was defined when the seminal parameters reached the minimum quality standards recommended by the Colégio Brasileiro de Reprodução Animal - CBRA (Henry & Neves, 1998).

In order to assess the semen, samples were collected through the artificial vagina method, in the period from the 20th to the 44th week of age. After that, the semen was placed in graduated plastic tubes and volume, aspect, concentration, wave motion, vigor, percentage of sperm cells with individual progressive motility, and spermatic morphology (minor, major and total defects) were assessed in accordance with Henry & Neves (1998). The aspect was classified by using a numerical score ranging from 1 to 3: 1 = watery, 2 = milky, and 3 = creamy (Lima et al., 2010). Sperm morphology was assessed through phase-contrast optical microscopy (Martins et al., 2006).

Measurements of live weight and scrotal circumference were carried out every fifteen days, from the 20th to the 44th weeks of age, by the same person, using scales and tape measures specific for this purpose.

To determine the serum testosterone levels, blood samples were collected into *vacutainer* tubes (4.0 mL) in the 20th, 28th and 38th weeks of age, through jugular venipuncture. Aliquots of serum obtained by immediate centrifugation (700 g) for 15 minutes were placed into *eppendorf* tubes and stored at –20ºC for subsequent hormonal dosage. Dosages were made by means of electrochemoluminescent immunoassay, using the Elecsys Testosterone Kit (Roche Diagnostic, Germany) with analytical sensitivity of 0.12 ng.mL^{-1} and centesimal precision and intra-assay coefficient of variation at 4.6%.

Statistical analysis was conducted by using the SAEG system version 9.1 (2007). The variables were subjected to tests of normality (Lilliefors) and homoscedasticity (Cochran & Bartlett), when it was verified the need for transformation of concentration, percentage of sperm cells with individual progressive motility, and abnormality spermatic data into logarithms. The variables concerning live weight, scrotal circumference, seminal parameters and serum testosterone (mean ± SE) were subjected to ANOVA and the means were compared by Duncan test ($P<0.05$). For determinations of correlations among live weight, scrotal circumference and semen parameters, the Spearman correlation was applied ($P<0.01$).

Results and Discussion

Although partial exposure of the penis was observed in some younger animals, the Anglo-Nubian goats raised under semi-intensive system showed penis debridement at 102.9 ± 15.4 days of age. The full sequence of sexual behavior (including mating capacity, ejaculation with viable sperm cells), which is understood as reaching puberty was observed, on average, at the 20th week (145.2 ± 9.7 days) of age. In the same age, the animals showed live weight and scrotal circumference of 25.75 ± 3.51 kg and 21.1 ± 1.64 cm, respectively.

Similar reports were made by Gauthier et al. (2001), working with Nigerian Dwarf goats raised under intensive system. The authors observed occurrence of puberty at about the 20th week of age, whereas Nishimura et al. (2000), in experiments with Japanese Tokara goats (also raised under intensive system) observed the occurrence of puberty in the 17th week of age. On the other hand, Madani & Rahal (1988) described the influence of the season of birth on the age of puberty in native Libyan goats, so that the animals born in winter were more precocious than those born in summer (22nd *vs.* 27th weeks of age, respectively). Therefore, it is evident that the onset of sexual activity in small ruminants may vary depending on breed, management system and birth date (Madani & Rahal, 1988; Ahmad & Noakes, 1996; Abi Saab et al., 1997; Almeida et al., 2007).

It is noteworthy that the gradual increase (P<0.05) of scrotal circumference occurred in alternating periods of significant growth and stability from the 20th to 44th weeks of age (Figure 1), similar to what was observed in native Brazilian goats (Eloy & Santa Rosa, 1998). Even after reaching sexual maturity, changes in scrotal circumference can occur in goats due to the influence of photoperiod, nutritional status and temperature (Coelho et al. ,2006; Almeida et al., 2007; Delgadillo et al., 2007).

As age increased, it has been verified a high positive correlation between live weight and scrotal circumference (r = 0.94). It was also found high positive correlation between age and live weight (r = 0.70) and also between age and scrotal circumference (r = 0.68). Similar to what was observed in the present study, Keith et al. (2009) working with Boer goats (32°N) and Raji et al. (2008) with White Borno goats (11ºN) found a high positive correlation (r = 0.78 and r = 0.82, respectively) between live weight and scrotal circumference. When analyzed as a whole, these findings reinforce the idea that, regardless of breed and latitude, the testicular growth is closely correlated to live weight and the age of goats.

Physical and morphological characteristics of semen (aspect, volume, wave motion, individual progressive motility, vigor, sperm concentration and total defects) were significantly influenced by age (P<0.01), thereby allowing the gradual evolution of the quality and the quantity of sperm within the period from the 20th to the 38th week of age, when the andrological parameters reached the minimum levels recommended by the CBRA (Henry & Neves, 1998).

The most significant increase (P<0.05) in sperm concentration was found in animals at 20th to 24th weeks of age (Table 1). According to Aguiar et al. (2006), such remarkable increase in the spermatogenic activity at the referred age results from the significant development of the seminiferous tubules and to the Sertoli cell differentiation.

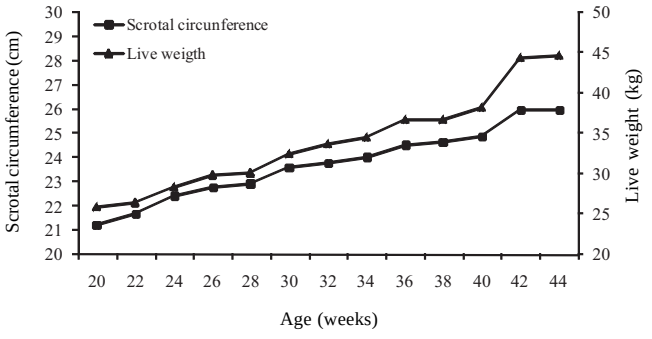

Figure 1 - Live weight and scrotal circumference in Anglo-Nubian goats raised in semi-intensive system from the 20th to the 44th week of age.

Table 1 - Seminal parameters regarding the period from the 20th to the 44th week of age of Anglo-Nubian goats raised in semi-intensive system.

Age (week)	Aspect*	Volume(mL)	Concentration ($\times 10^9$ sperm cells.mL^{-1})
20th	2.10 ± 0.94b	0.38 ± 0.05c	1.33 ± 0.64c
22nd	2.30 ± 0.82ab	0.36 ± 0.07c	2.12 ± 0.72bc
24th	2.50 ± 0.57ab	0.44 ± 0.14c	2.59 ± 0.12ab
26th	2.67 ± 0.65ab	0.45 ± 0.07c	2.54 ± 0.32ab
28th	2.50 ± 0.81ab	0.43 ± 0.07c	2.73 ± 0.36ab
30th	2.57 ± 0.75ab	0.48 ± 0.09c	2.76 ± 0.46ab
32nd	2.71 ± 0.46ab	0.54 ± 0.07bc	2.91 ± 0.34ab
34th	2.91 ± 0.30a	0.53 ± 0.07bc	3.19 ± 0.35ab
36th	3.00 ± 0.00a	0.61 ± 0.11abc	3.51 ± 0.27a
38th	3.00 ± 0.00a	0.65 ± 0.13abc	3.55 ± 0.27a
40th	3.00 ± 0.00a	0.63 ± 0.09abc	3.63 ± 1.10a
42nd	3.00 ± 0.00a	0.75 ± 0.13ab	3.62 ± 0.34a
44th	3.00 ± 0.00a	0.96 ± 0.04a	3.54 ± 0.14a

Means followed by different letters in the column differ from each other (P<0.05) by Duncan test.
* 1 - watery, 2 - milky, 3 - creamy.

The values respecting the correlation between sperm concentration and the other parameters analyzed were variable and positive, with high and moderate values for age, weight, aspect, wave motion, individual progressive motility and vigor (P<0.01). No significant correlation (P>0.01) could be verified between sperm concentration and the ejaculate volume.

Along with the increasing concentration, the ejaculate volume has progressively increased with advancing age (P<0.05), while it corresponded to 0.38 ± 0.05 mL at the 20th week of age and to 0.96 ± 0.09 mL at the 44th week of age (Table 1). Similar findings were reported by Abi Saab et al. (1997), who observed a mean ejaculate volume of 0.5 ± 0.1 mL for Lebanese Baladi goats at the 23rd week of age.

The ejaculate volume observed in the present study showed positive correlations (P<0.01) with age, live weight and scrotal circumference (r>0.35). No significant correlation with other variables could be noted (r<-0.13).

According to Tutida et al. (1999) and Santos & Simplicio (2000), environmental factors such as nutritional status, seasonal variations, method of semen collection and ejaculatory frequency can influence the activity of accessory sex glands, which are responsible for 95% of the total volume of ejaculate.

The aspect of semen showed differences as age advanced (P<0.05); it was predominantly watery and milky in the period from the 20th to the 32nd week of age whereas all ejaculates observed from the 36th week of age were creamy (Table 1). Aspect showed a high positive correlation (P<0.01) with sperm concentration (r = 0.57) and moderate correlation with wave motion (r = 0.44) and individual progressive motility (r = 0.38). The significant correlation

between aspect and concentration was reported by Gonçalves et al. (2002), who found that the watery aspect of semen resulting from the low sperm concentration was inadequate for artificial insemination.

Wave motion increased from 1.81 ± 0.54 in the 20th week to 4.6 ± 0.24 in the 44th week of age (Figure 2) whereas a significant increase in this characteristic could be observed from the 20th to the 22nd week of age (P<0.05), when the minimum score (≥3.0) recommended by CBRA was reached (Henry & Neves, 1998). Wave motion is a physical characteristic of semen and it depends on sperm concentration, vigor and individual progressive motility; thus, the moment of the remarkable evolution of this characteristic can be understood as a reference of when puberty is reached.

The vigor (displacement force) of cells with progressive motility was the characteristic that showed the smallest variation, as it could be noted no differences (P>0.05) caused by increasing age. Despite the extreme values of 3.7 ± 0.54 and 5.0 ± 0.0 in the 20th and 44th weeks of age, the ≥ 4.0 score was reached from the 24th week of age (Figure 3).

The individual progressive motility had a significant variation with advancing age: 48.0 ± 10.52% in the 20th week and 82.0 ± 3.74% in the 44th week of age (P<0.05). The minimum score of 70%, which is recommended by the CBRA (Henry & Neves, 1998), was achieved from 38th week of age (Figure 4). The individual progressive motility showed moderate and negative correlation with sperm defects (P<0.01). As the reduction of sperm defects occurred, the seminal feature evolved gradually until reaching the levels recommended by the CBRA (Henry & Neves, 1998). Fernandes et al. (2009) have recently described the high incidence of abnormalities in the intermediate segment and tail of the sperm as the responsible factor for the low sperm motility. In addition, Horn et al. (2002) have pointed out that the epithelium of the epididymis was responsible for the selection and phagocytosis of sperm cells with certain defects; such process becomes more efficient as the reproductive maturity is reached. Thus, the high value of individual progressive motility (≥70%) can be interpreted as an indicative that sexual maturity has been reached.

The number of defective cells reduced from 32.17 ± 5.82% in the 20th week to 8.80 ± 2.96% in the 44th week (P<0.05). From the 20th to the 24th week of age, the major defects accounted for 65% of the overall defects. The most frequent major defects at that age were decapitated spermatozoa, acrosome defects, "dags" and proximal cytoplasmic droplets. In the assessment of overall defects, it was verified that ejaculates reached the quality recommended by the CBRA (<20%) in the 26th week of age (Table 2). According to Pacheco et al. (2007), the major

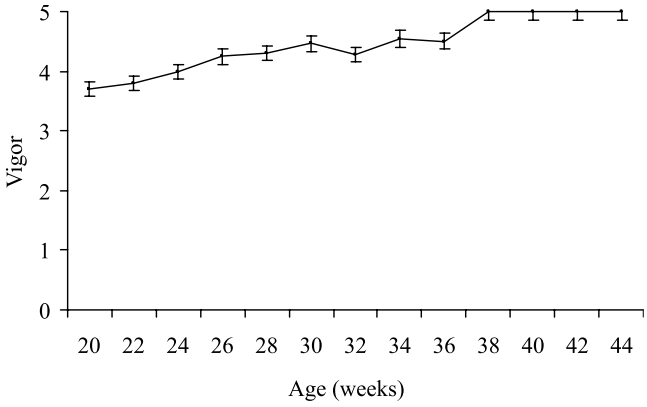

Figure 3 - Vigor of sperm cells in Anglo-Nubian goats raised in semi-intensive system in the period from the 20th to the 44th week of age.

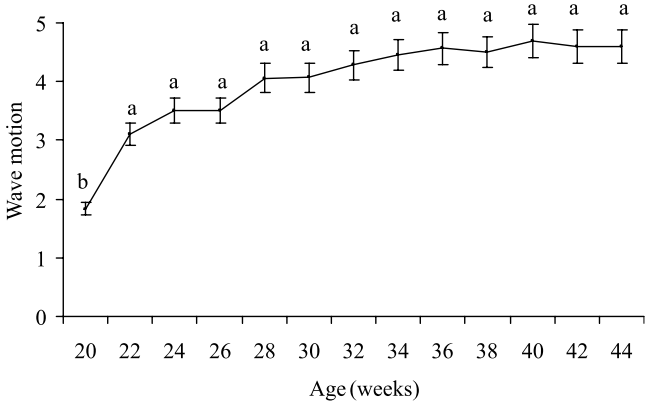

Figure 2 - Wave motion in Anglo-Nubian goats raised in semi-intensive system in the period from the 20th to the 44th week of age.

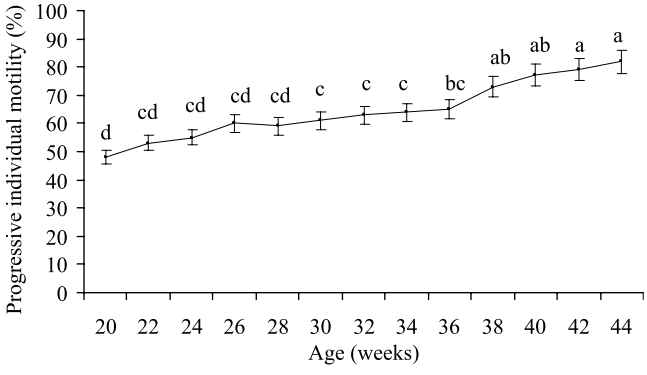

Figure 4 - Progressive individual motility in Anglo-Nubian goats raised in semi-intensive system within the period from the 20th to the 44th week of age.

Table 2 - Morphological characteristics of semen in Anglo-Nubian goats raised under semi-intensive system, from the 20th to the 44th week of age

Age (weeks)	Major defect (%)	Minor defect (%)	Total defect (%)
20th	18.92 ± 3.47a	13.25 ± 2.63a	32.17 ± 5.82a
22nd	15.90 ± 5.99a	9.80 ± 3.61ab	25.70 ± 9.58ab
24th	14.75 ± 1.38ab	6.50 ± 2.02ab	21.25 ± 2.49ab
26th	12.75 ± 1.98ab	6.83 ± 1.35ab	19.58 ± 2.35ab
28th	11.60 ± 1.26ab	5.33 ± 0.85bc	16.93 ± 1.56ab
30th	10.86 ± 2.73ab	5.14 ± 1.45bc	16.00 ± 3.62bc
32nd	11.31 ± 1.15ab	4.87 ± 2.58bc	16.18 ± 2.72bc
34th	10.73 ± 2.70ab	2.64 ± 0.75cd	13.37 ± 2.82c
36th	10.17 ± 2.86ab	2.33 ± 0.49cd	12.50 ± 2.84c
38th	10.40 ± 0.98ab	3.40 ± 1.50cd	13.80 ± 2.03c
40th	10.70 ± 2.96ab	2.50 ± 0.67cd	13.20 ± 3.48c
42nd	6.90 ± 1.31b	1.60 ± 0.58d	8.50 ± 1.40d
44th	7.40 ± 3.14b	1.40 ± 0.60d	8.80 ± 2.96d

Means followed by different letters in the column differ from each other (P<0.05) by Duncan test.

defects are those which were originate in the tests or in the epididymis, and may be related to defective spermatogenesis, which was certainly caused by reproductive immaturity.

There has been a considerable reduction in the minor defects within the period from the 20th to the 34th week of age (P<0.05) and a subsequent stabilization of values. The most frequent minor defect was the bent tail, followed by coiled tail and distal cytoplasmic droplets. According to Barth & Oko (1989), sperm tail pathologies (bent or coiled) may result from failures in thermoregulation, testicular degeneration, hypoosmotic conditions or failures in epididymal transit. Just like what was observed by Folhadella et al. (2006) in the bovine species, the high prevalence of tail pathologies in this study, such as the distal cytoplasmic droplet can be associated with sexual immaturity.

Except for the volume, the correlations between the morphology and the parameters evaluated were negative and significant (P<0.01), whose lowest and highest correlations involved vigor (r = -0.27) and wave motion (r = -0.44), respectively (Table 3).

The serum testosterone levels varied according to age: low values in the 20th week, high values in the 28th week and again low values in the 38th week of age (P<0.05). In the 20th and 38th weeks of age, serum levels ranged from 0.4 to 5.4 ng.mL^{-1} whereas in the 28th week, levels ranged from 2.6 to 14.2 ng.mL^{-1} (Table 4). The results were very similar to the findings by Eloy & Santa Rosa (1998), who found an increase in testosterone levels from the 10th to the 18th week of age, with values of 3.02 and 5.52 ng.mL^{-1}, respectively, when monitoring native Brazilian goats.

According to Nishimura et al. (2000) and Moura et al. (2002), the reactivation of Leydig cells, associated with the proliferation of germ cells, is an essential physiological event to achieve puberty. Nishimura et al. (2000) reported that the full development of the seminiferous epithelium in Japanese native goats ends around the 26th week of age. Thus, the increased testosterone serum levels observed in this study between the 20th and the 28th week of age can be understood as a reflection of the increased activity of Leydig cells, which results from the maturation of the hypothalamic-pituitary-testicles axis, therefore acting as a biochemical marker that indicates when puberty is reached.

On the other hand, the subsequent reduction of serum testosterone levels after reaching the peak observed in this study was likewise observed by Eloy & Santa Rosa (1998) in native Brazilian goats, whose values have decreased from 5.52 to 2.78 ng.mL^{-1}, from the 18th to the 28th week of age, respectively. According to Amann & Shanbacher (1983),

Table 3 - Coefficients of correlation among age, live weight, scrotal circumference, and semen parameters in Anglo-Nubian goats raised under semi-intensive system

Sperm Characteristics	Body weight	Scrotal circumference	Volume	Aspect	Wave motion	Progressive individual motility	Vigor	Concentration	Total defects
Age	0.72*	0.68*	0.44*	0.40*	0.43*	0.30*	0.29*	0.40*	-0.40*
Body weight	-	0.94*	0.38*	0.23*	0.27*	0.23*	0.19*	0.31*	-0.30*
Scrotal circumference		-	0.37*	0.20*	0.28*	0.19*	0.20*	0.26*	-0.34*
Volume			-	-0.09	-0.03	-0.05	-0.01	-0.13	-0.02
Aspect				-	0.44*	0.38*	0.28*	0.57*	-0.37*
Wave motion					-	0.58*	0.48*	0.58*	-0.44*
Progressive individual motility						-	0.61*	0.47*	-0.35*
Vigor							-	0.28*	-0.27*
Concentration								-	-0.39*

* P<0.01.

Table 4 - Serum testosterone concentrations for Anglo-Nubian goats at different ages raised in semi-intensive system.

	Testosterone level (ng/mL^{-1})		
Age (week)	Minimum	Maximum	Mean
20^{th}	0.9	4.1	2.7 ± 1.4b
28^{th}	2.6	14.2	8.5 ± 4.6a
38^{th}	0.4	5.4	2.2 ± 2.2b

Means followed by different letters in the column differ from each other (P<0.05) by Duncan test.

high peripheral concentration of testosterone through negative feedback on the hypothalamus reduces the production of GnRH, which reduces the release of LH by pituitary through the cascade effect, thus reducing the stimulus on Leydig cells and, consequently, the plasma levels of testosterone.

Conclusions

Serum testosterone concentration and andrological parameters of Anglo-Nubian goats raised in semi-intensive system are closely related to age. Puberty is reached in the 20^{th} week of age whereas sexual maturity is reached in the 38^{th} week of age, and the percentage of spermatozoa with progressive motility is the latest seminal parameter.

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