

BIOMETRY AND TESTICULAR GROWTH INFLUENCED BY NUTRITION ON PREPUBERTAL PELIBUEY LAMBS

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ABSTRACT: The growth and testicular development was studied in 48 Pelibuey male lambs 76.6 ± 3.0 days of age and 12.7 ± 1.9 kg body weight (BW), two groups were designed ($n=24$). 1: Intensive rotational grassing (IRG), 2: Intensive rotational grassing plus nutritional supplement (IRGS). BW was recorded every 15 days from 75 days of age to the onset of puberty. The animals grazed on *Panicum maximum*. IRGS received a concentrate with 15% of protein. The testicular biometry included scrotal circumference (SC) and testicular volume (TV). Blood samples were collected each 15 days from 90 to 190 days of age for evaluate the testosterone concentrations. BW, SC and TV at histological puberty was higher in IRGS than IRG; 22.5 ± 1.5 vs. 16.06 ± 1.5 kg, 22.0 ± 1.0 vs. 12.2 ± 1.5 cm, 60.5 ± 1.7 vs. 12 ± 3.5 cm³ respectively ($P < 0.05$) with an average age for the two groups of 162 ± 7.0 days. The correlation coefficient (R) was higher ($P < 0.05$) for SC vs BW than age vs BW (0.884 vs 0.816) and the TV vs. BW than TV vs. age (0.849 vs. 0.777) in the IRGS; the IRG showed lower R for the same comparisons ($P < 0.05$). Seminiferous tubules showed lumen by day 142, spermatids and spermatozooids by day 171 for IRGS, meanwhile in the IRG only showed gonocytes and Sertoli cells. Testosterone concentrations reached a peak (2.5 ng/ml) at 168 days of age for the IRGS meanwhile the IRG showed lower levels than 0.05 ng/ml. Testicular development and testosterone concentrations depends more on BW than age; and they are modified by the nutritional management in prepuberal male lambs.

Key words: Testis Development, Puberty, Nutrition, Lambs

INTRODUCTION

In sheep, as in any other domestic species, the reproductive performance is considered as the most important in terms of economic value (Bilgin et al., 2004). There are four factors that can determine performance: (1) genetic merit, (2) physical environment, (3) nutrition and (4) management; it has been suggested that the nutritional factors are the most important in terms of their direct effects on reproduction, while the other factors are considered as having only irregular influence (heat stress, pre weaning management, for example). Adequate nutrition can stimulate biologically mediocre individuals to attain their genetic potential, diminish the negative effect of a physically hostile environment and minimize the effects of deficient management techniques (Fourie et al., 2004). Therefore, appropriate nutritional management is a decisive factor for the successful reproduction of a flock (Fernández et al., 2004); indeed, the energy deficiency caused by a low level of ingestion or by excessive utilization diminishes the secretion of gonadotrophines in both sexes of many species, humans included, but reestablishing normal feeding patterns generally reverses any deficit of hormones (Brown, 1994; Blache et al., 2000; Bielli et al., 2002). Testicular size and sperm production may be affected by changes in protein ingestion, even when such changes exceed the maintenance requirements (Fernández et al., 2004). There appears to be no reciprocal effect from changes in the secretion of testosterone, and this tends to strengthen the hypothesis that the connection between protein ingestion and reproduction is based on an effect not dependent on GnRH (Hötzel et al., 1998; Fernández et al., 2004). However, it has been established that the regulation of testicular growth through nutrition also includes a route that is dependent on GnRH (Blache et al., 2000). Numerous studies (Blache et al., 2000; Fourie et al., 2004) have shown that spermatogenesis in rams is sensitive to increments in protein ingestion. This effect has been associated with an increase in testicular size as reflecting an increase in the volume of the

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seminiferous epithelium and in the diameter of the seminiferous tubuli (Saab et al., 1997; Hötzel et al., 1998); thus, the size of the testis is directly related to the potential of sperm production.

In the case of rams, changes in body weight (BW) are directly correlated with testicular growth and regression (Murray et al., 1990). The size of the testis is considered as the most adequate criterion, from the physiological, genetic and practical perspective, for improving the reproductive performance of female descendants; this indirect criterion of selection is dependent on the heritability and the genetic correlation between testicular size and female reproductive traits (Matos et al., 1992). It has been observed that males with bigger testis tend to produce daughters that reach puberty at an earlier age and liberate more ovules during each estrous period (Söderquist and Hultén, 2006).

Concerning the nutrition of growing rams, it has been reported that the reproductive functions in young animals seems to be more susceptible to restrictions in energy and protein than those in adults; furthermore, severe nutritional restrictions can result in permanent damage to gonad and neural tissues (Brown, 1994). Recently, Bielli et al. (2002) reported that, starting in the uterine stage, deficient nutrition during pregnancy in the ewe may reduce testicular development in the newborn lamb, although poor nutrition and the ingestion of toxic substances can have a greater effect on testicular development and spermatogenesis. The reproductive system possesses considerable regenerative capacity, unless there have been severe and prolonged dietary deficiencies (Brown, 1994). Post-weaning nutritional management strongly influences weight increase in rams, which has been found to be associated with testicular growth and the onset of puberty in rams of the Menz breed (Mukasa-Mugerwa and Ezaz, 1992); besides, measuring the scrotal circumference (SC) is an essential characteristic of andrological evaluation if we take into account that testis size varies according to the breed, the age and the season of the year (Söderquist and Hultén, 2006). Therefore, measuring the size of the scrotum as a criterion for early selection in small ruminants (Mukasa-Mugerwa and Ezaz, 1992) makes it possible to measure the performance of these rams bred under different nutritional strategies and, consequently, to evaluate their diets as indicators of precocity. The object of this study was to determine the correlations between age and BW with testicular biometry, in addition to measure the testosterone concentrations and histologically determine the presence of spermatozooids in prepubertal Pelibuey rams under two nutritional regimes: intensive rotational grazing and intensive rotational grazing plus nutritional supplement.

MATERIALS AND METHODS

Location

This research project was carried on for one year at the Postgraduate College – Veracruz Campus, situated at 19° 11'45" N and 96° 19'03" W (GPS 12, Garmin International Inc.), in a warm climate with rains in summer.

Experimental animals

Forty-eight Pelibuey male lambs aged 76.6±3.0 days with BW 12.7±1.9 kg, born from single or double parturition with twins of either sex, were randomly assigned to either of two experimental groups (n=24). Group 1: intensive rotational grazing (IRG), Group 2: intensive rotational grazing plus nutritional supplement (IRGS); each group consisted of 8 weaned lambs during each of the following three climatological seasons of the year: rainy: August to November; windy: December to March; dry: April to July. The BW was recorded every 15 days from 75 days of age to the onset of puberty in any lamb identified with the aid of histological techniques.

Feeding

The animals grazed in established meadows of Tanzania grass (*Panicum maximum*) for 7 days, followed by 21 days of rest for each meadow. The IRGS group received a commercial concentrate for lambs (Campi corderos®, Veracruz, Mexico) with 15% of crude protein, the supply of which was adjusted to be equivalent to 1.5% of the BW recorded on the scales every 15 days during the 3 seasons of the year.

Morphological evaluations

A biometry was performed in the testicular region as follows: (1) The scrotal circumference (SC) was obtained by forcing both testicles to descend completely into the scrotum (Matos et al., 1992), with the aid of a flexible measuring tape placed at the maximum transverse diameter encountered in the scrotal sac (Bielli et al., 2000). The testicular volume (TV) was calculated from the biometry performed at the greater and lesser axes of each testis with the aid of a vernier graduated in millimeters; for this calculation, the following equation proposed by Steger and Wrobel (1994) was applied:

$$TV = 1/6 * (\pi) * a * b * 0.945 \text{ (Equation 1)}$$

In which:

TV=testicular volume

$\pi=3.1416$

a=testicular width

b=testicular length

The testicular biometry was performed at intervals of 15 days, starting from the 75th day of age in all the animals of both feeding groups and in all seasons of the year.



Histological evaluations

In order to obtain testicular samples, a hemicastration by means of lateral approximation was performed at intervals of 15 days starting on day 90 of age. For these evaluations only one animal from each feeding system and season of the year was employed. The animals were tranquilized with a sedative consisting of xylazine (Rompun® Bayer) and ketamine (Ketamina® Cheminova). After castration, a sample of tissue in the form of a cube with a volume of 8-10 mm³ was taken from each of the three transverse sectors at the greater testicular axis, and then the samples were fixed in a modified Davidson solution (Latendresse et al., 2002) during 48 hours. Thereafter, they were washed in ethyl alcohol at 70% for two hours on two occasions; in this last solution they were processed to obtain histological sections of 5 µm in thickness for their subsequent staining with hematoxylin and eosin. Once the stained laminae had been obtained, they were examined under a microscope (Leica microscope 40X) for the cellular development and structures, such as: (1) the lumen, (2) spermatocytes, (3) spermatids or spermatozooids, so as to have a histological basis for determining the onset of puberty, such as the initial liberation of spermatozooids from the seminiferous epithelium (Herrera-Alarcón et al., 2007). The images were taken with the help of a Motic 1.3 Mpxel digital camera.

Endocrinological evaluations

Blood samples from the jugular vein were collected in tubes having an anticoagulant (EDTA) at 15-day intervals between 90 and 210 days of age during the rainy season. The samples were centrifuged at 2000 g for 10 minutes, and the supernatant plasma was recovered and frozen at -20 °C for its quantification. The testosterone concentration was determined by solid phase radioimmunoassay with a commercial antibody kit marked with I¹²⁵, and the reading was taken with an automatic gamma counter (2470 WIZARD2, Perkin Elmer) (Ungerfeld and Silva, 2004).

Statistical analysis

The data were recorded on an electronic calculation sheet. The age (in days) and the body weight were used as independent variables, the dependent variables were: scrotal circumference (cm) and testicular volume (cm³). To measure the degree of association, a simple exponential equation was employed:

$$f = a * e^{(b * x)} \text{ (Equation 2)}$$

In which:

f = dependent variable (SC cm or TV cm³)

a = value of the body weight to the maturity (estimated)

e = 2.7182

b = value of curve integral

x = age (days) or weight (kg)

To describe the growth curve of the lambs, a Gompertz mathematical model was used:

$$BW = a * e^{(-e^{-(x - x_0)})/b} \text{ (Equation 3)}$$

In which:

BW = body weight (kg)

a = value of the body weight to the maturity (estimated)

b = inflection point in days (age when maximum growth is observed)

x = age (days)

x₀ = age at initial curve inflection

To measure the relationship between scrotal circumference (cm) and testicular volume (cm³), the following equation was used:

$$SC = y_0 + a * \ln(x) \text{ (Equation 4)}$$

In which:

SC = scrotal circumference (cm)

y₀ = value of scrotal circumference (cm) when testicular volume (cm³) is zero

a = integral of equation

x = testicular volume (cm³)

RESULTS AND DISCUSSION

The results show that the quality of the diet is a determining factor in body development (Fig. 1e) and hence in testicular growth (Figure 2); this is similar to observations Fourie et al. (2004), reported for young Dorper rams, in which a better diet with greater energy and protein complementation was able to improve reproductive performance. In previous work with adult rams of the Assaf breed (Fernández et al., 2004), statistical differences in testicular size and sperm production were found upon comparing diets with different protein contributions: the values recorded for SC and TV were lower in sheep that consumed barley chaff and a nutritional supplement with 13.6% of crude protein (CP) than in those that received supplements containing 16.4% and 20.5% of CP concentrate (Fernández et al., 2004). Bielli et al. (1999), failed to find any significant effects on testicular dimensions upon improving the forage or increasing the protein in the diet for rams of the Corriedale breed. In



Merino rams, however, it was found that testicular dimensions responded better to the ingestion of digestible energy than to the availability of CP in the diet, which produced only a marginal effect (Murray et al., 1990). In the present study, the experimental animals that consumed a better diet (IRGS group) showed more gonadal growth (Table 1); this made it possible for them to reach histological puberty at ages oscillating between 156 and 177 days during all the climatic seasons studied. The BWs recorded were between 22 and 23 kg, which are similar to those reported by Herrera-Alarcón et al. (2007) for rams of the Blackbelly breed.

Table 1. Biometric descriptors of Pelibuey rams kept in conditions of intensive rotational grazing (IRG), or rotational grazing plus nutritional supplement (IRGS) at the moment of histological puberty were determined in the IRGS in different seasons of the year

Biometric Descriptor	Intensive Rotational Grazing Plus Supplement (IRGS)			Intensive Rotational Grazing (IRG)		
	Rainy	Windy	Dry	Rainy	Windy	Dry
Body weight (kg)	22.5	23.0	22.0	15.5±1.05	16.3±0.98	16.4±1.0
Scrotal circumference (cm)	23.0	22.0	21.0	9.4±0.4	13.1±0.3	14.2±0.4
Testicular volume (cm ³)	62.0	61.1	58.4	1.9±0.2	12.2±1.95	22.6±2.35
Age of puberty (days)	177	156	166	167±6.0	145±7.0	164±9.0

Note: Values for the IRGS were obtained from the first animal to attain histological puberty; the number of animals in the IRG was 4.

It has been reported that, in rams of the Chios, Serres and Karaguniki breeds born in the month of October, the first spermatozooids appear in the ejaculate around 142 days of age, when their weight averages 35 kg (Alexopoulos et al., 1991). Puberty in rams is considered to begin at the first mounting with ejaculation and appears to be associated more with BW than with chronological age (Belibasaki and Kouimtzis, 2000). Fernández et al. (2005) reported that the SC was smaller in rams fed with a nutritional supplement having low protein content than in those receiving a supplement high in protein, which improves the performance in the animals during the mating period and accelerates testicular growth. In the IRG group, differences occurred in the testicular biometry during the various seasons of the year (Table 1); these may be attributable to fluctuations in the quality of forage during the experimental phases in the field.

Mukasa-Mugerwa and Ezaz (1992) found significant reproductive variations due to effects arising from the season of birth, nutrition level and weight at weaning. In the present study, the SC at the beginning of puberty showed an average of 22±1 cm for the IRGS group in all seasons. In a study carried out by Avellaneda et al. (2006), the SC at the onset of puberty was 23.8, 22.9, 20.8 and 23 cm for the Romney Marsh, Mora Colombian, Creole and Hampshire breed, respectively. In the present study, TV was 60.5±2 cm³, at an age of 166±11 days and with a BW of 22.5±1 kg (Table 1). Similar values were found when evaluating the testicular characteristics of Ile de France x Akkaraman rams, with average SC measurements of 23.8±0.55 cm and TV of 51.7±2.76 and 57.8±3.76 cm³ for the left and right gonads, respectively (Mert et al., 2009). Nevertheless, these values are lower than those reported by Herrera-Alarcón et al. (2007) from their work with Blackbelly rams aged 172 days; they recorded a value of 33.5 cm for the SC in rams of mature age. According to Söderquist and Hultén (2006), the SC in rams of the Gotlandic breed measured 28.9±1.9 cm at an age of 170±9 days, while the BW was found to be 53.5±7.0 kg for Merino rams at 73 and 143 days, Steger and Wrobel (1994) reported TV of 7.18 and 149.13 cm³, respectively. In the present work, a marked difference was found to exist upon analyzing the TV of both gonads and comparing ages; a correlation coefficient of R=0.777 was obtained for the IRGS group, and R=0.092 for the IRG group (Table 2, Figure 1c).

Table 2 - Adjustments in the testicular biometry performed on Pelibuey rams kept in conditions of rotational grazing (IRG), or rotational grazing plus nutritional supplement (IRGS)

	Intensive Rotational Grazing plus Supplement (IRGS)				Intensive Rotational Grazing (IRG)			
	a	b	R	R ²	a	b	R	R ²
1) Body weight (kg)								
Scrotal circumference (cm)	4.08±0.220	0.070±0.003	0.884	0.780	4.27±0.332	0.06±0.005	0.711	0.503
Volumen testicular (cm ³)	0.44±0.126	0.023±0.013	0.849	0.720	0.30±0.129	0.24±0.021	0.653	0.423
2) Age								
Scrotal circumference (cm)	5.81±0.30	0.006±0.004	0.816	0.664	9.44±0.73	0.001±0.0006	0.189	0.029
Testicular volumen (cm ³)	1.91±0.41	0.020±0.001	0.777	0.602	7.00±3.35	0.003±0.003	0.092	0.002
Body Weight (kg)	31.82±8.28	127.37±53.0	0.770	0.593	15.27±0.47	23.36±11.92	0.340	0.115
3) Testicular Volume (cm ³)								
Circunferencia escrotal	3.35±0.07	5.23±0.211	0.962	0.926	2.75±0.09	6.41±0.188	0.921	0.848

a, b = adjustment parameters for the mathematical model; y₀ = value of intercept, R = correlation coefficient, R² = determination coefficient.

In Menz lambs, the age and the BW at the start of puberty were 288±6 days and 19.3±0.4 kg, respectively, the SC being 21.5±0.3 cm (Mukasa-Mugerwa and Ezaz, 1992). Regarding other breeds, the BW at puberty was 31.2, 29.0, 26.9 and 29 kg, at the ages of 235, 214, 231 and 196 days for the Romney Marsh, Mora Colombian, Creole and Hampshire breed, respectively (Avellaneda et al., 2006). In a study carried out under semi-cold and semi-humid climatological conditions in Mexico, Pelibuey lambs reached puberty at 144.07±8.43 days with a BW of 32.6±3.94 kg and an SC of 25.86±2.24 cm, on a diet that contained 2.85 Mcal of metabolized energy/kg



(Valencia et al., 2005). By utilizing the point of inflection on the growth curve for the SC, ages of 140 and 152 days at puberty were determined for the Redkaraman and Awassi breeds, respectively (Emsen, 2005); whereas for lambs of the Friesland, Karagoniki, Chios and Serres milch sheep breeds, puberty was determined as starting at 170,187, 189 and 209 days, respectively (Belibasaki and Kouimtzis, 2000).

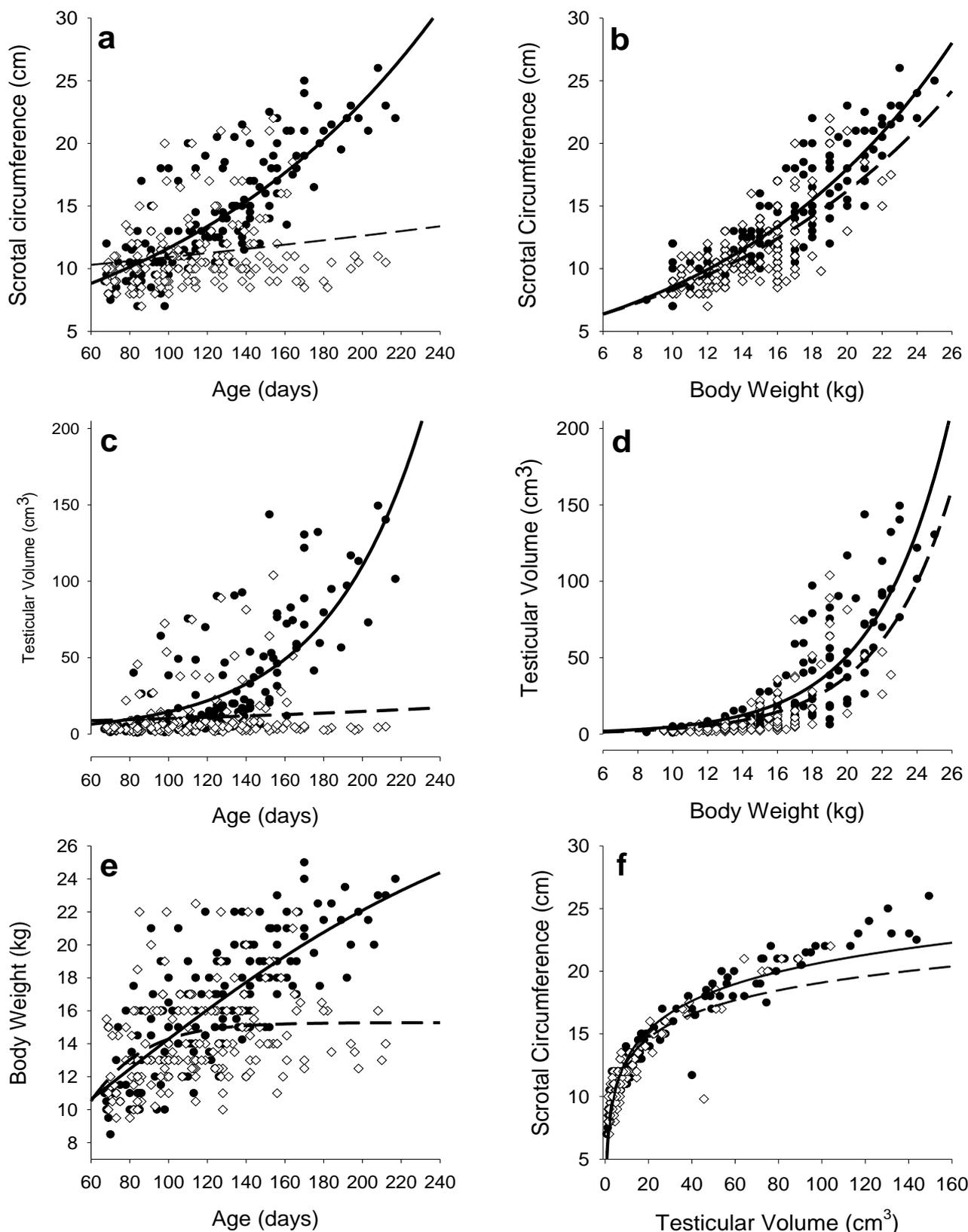


Figure 1 - Adjusted curves of the testicular biometry in regard to age (a,c) and body weight (b,d). Solid line: Intensive Rotational Grazing Plus Supplement (IRGS); Broken line: Intensive Rotational Grazing (IRG). ●IRGS, ◇IRG. e: age vs. bodyweight relationship; f: testicular volume vs. scrotal circumference relationship



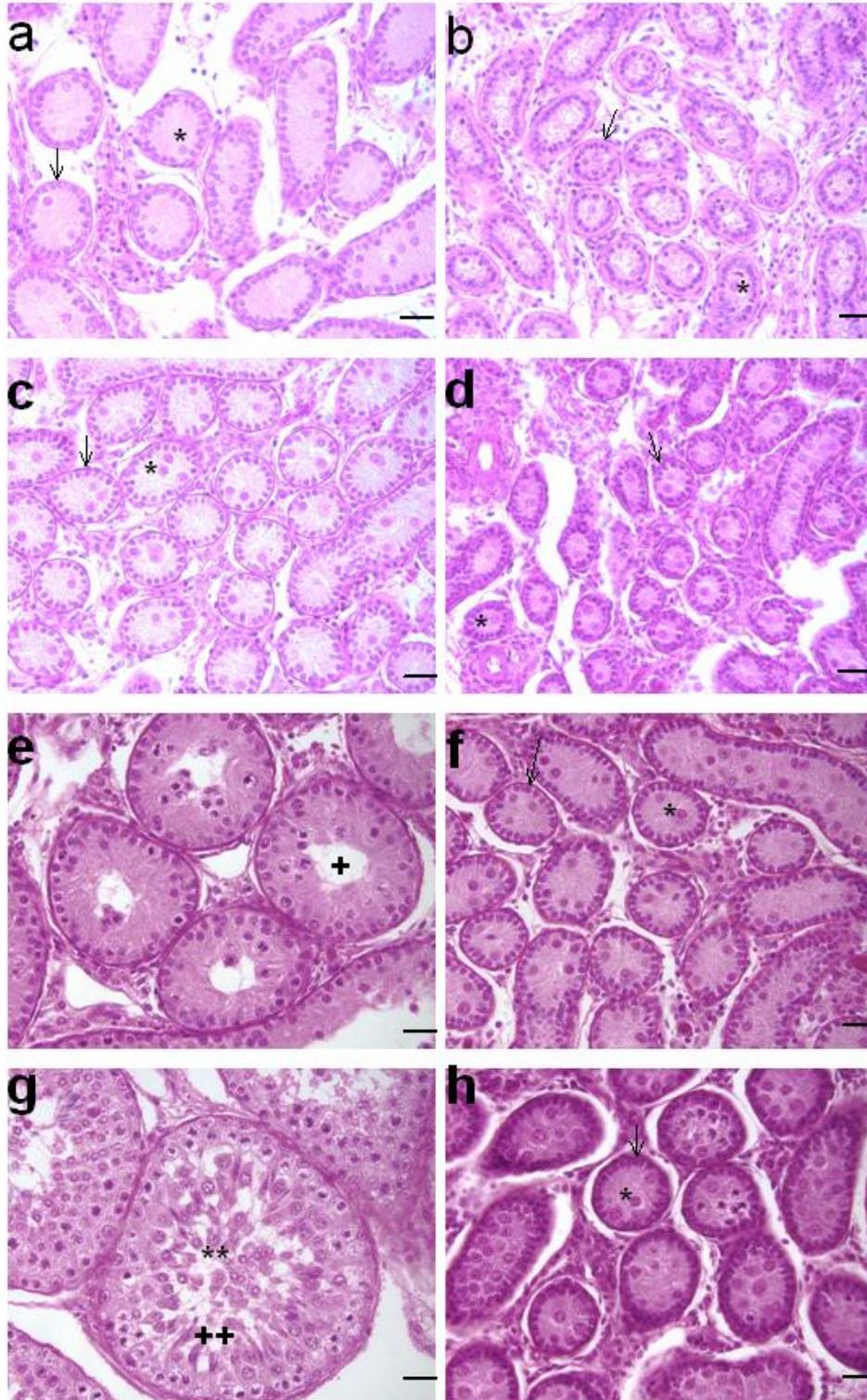


Figure 2 - Findings in the germinal testicular epithelium (40x). a,c,e,g: Intensive Rotational Grazing plus Supplement (IRGS); b,d,f,h: Intensive Rotational Grazing (IRG). a,b: age 83±2 days; c,d: age 113±1 days; e,f: age 142±1 days; g,h: age 171±5 days; mean ± standard deviation. *Prespermatogonium (Gonocyte); →Sertoli cells; +Lumen of seminiferous tubule; **Spermatides; ++Spermatozooids. The bar in the lower right corner of all pictures measures 25 µm

In this study, the correlation coefficient between BW and SC was $R = 0.884$ for the IRGS (Table 2); this coincides with the correlation value ($R=0.86$) reported for the testicular growth and changes in the BW of Merino lambs (Murray et al., 1990) and that of $R=0.85$ for Menz lambs (Mukasa-Mugerwa and Ezaz, 1992). Considering the high correlation between the BW and the SC, it is necessary to evaluate puberty on the basis of body and gonadal development rather than age ($R=0.816$), since the SC is a direct indicator of sperm quality (Avellaneda et al., 2006). The correlation between BW and SC for the IRG was $R=0.711$ in the present work (Table 2).

Values of $R^2=0.780$ and $R^2=0.503$ were recorded as determination coefficients for the IRGS and the IRG, respectively (Table 2, Figure 1b), and these reinforce the finding that testicular measurements increase progressively and are better correlated with the BW than with the age (Salhab et al., 2001).

The correlation value for SC vs TV was $R=0.921$ for the IRG and $R=0.962$ for the IRGS. In lambs from the Ile de France x Akkaraman breeding, the correlation between the left SC and TV was $R=0.84$, and $R=0.90$ for the right (Mert et al., 2009). For these reproductive variables, determination coefficients of $R^2=0.926$ and $R^2=0.848$ were found for the IRGS and the IRG, respectively in the present study (Table 2, Figure 1f).

For the variables SC vs age, the correlation values were $R=0.189$ for the IRG and $R=0.816$ for the IRGS. In Menz lambs, the SC showed a high correlation with the age ($R=0.83$), although in this case it was also influenced by the nutritional level (Mukasa-Mugerwa and Ezaz, 1992). The results obtained confirm that when the ingestion of protein is increased above the requirements for maintenance and growth, puberty and fertility can be attained at an earlier age in small ruminants (Saab et al., 1997). In a study that included the Pelibuey breed, the SC and the age at puberty presented a correlation value of $R=0.59$ (Valencia et al., 2005). In the present study, values of $R^2=0.664$ and $R^2=0.029$ were founded for the IRGS and the IRG, respectively (Table 2).

As to hormonal activity, the values of testosterone for the IRGS group during the rainy season reached their maximum at 177 days of age (Figure 3) with 2.44 ± 0.61 ng/ml, which coincides with the onset of histological puberty; this synchronization is comparable to finding an elevation in testosterone of 0.78 ng/ml at 32 weeks, coincident with puberty (Avellaneda et al., 2006) in different ovine breed. Both findings confirm the stipulation by Herrera-Alarcon et al. (2007), affirming that testosterone values may be used as possible indicators of puberty in ovine males.

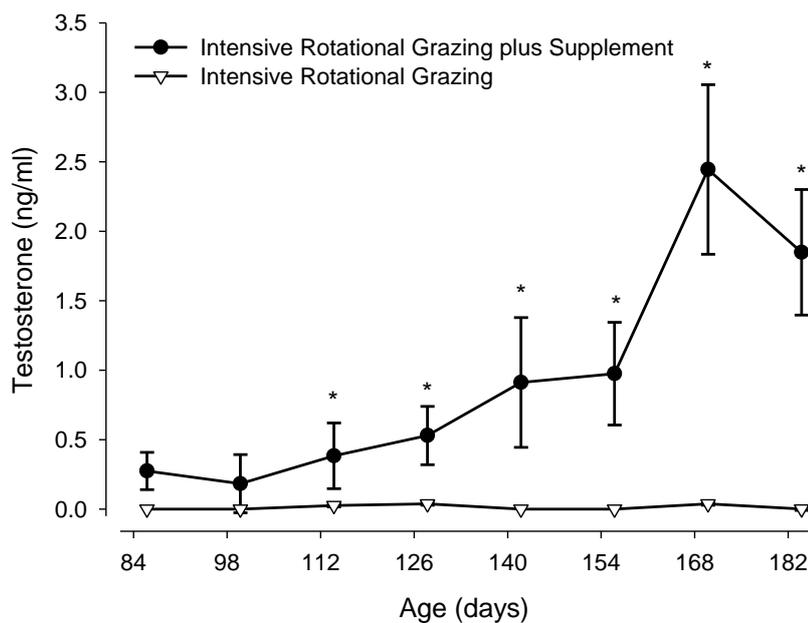


Figure 3. Testosterone concentrations (ng/ml) in serum of Pelibuey rams under conditions of Intensive Rotational Grazing plus Supplement (●) and Intensive Rotational Grazing (▽) (* $P<0.05$)

CONCLUSIONS AND IMPLICATIONS

Testicular development depends more on BW than on age, this being a reflection of the nutritional management under which the rams develop. Testicular measurements can be used as a tool for detecting animals that have been raised on nutritionally poor diets, although exists a considerable margin in the genetic plane that should be taken into account when making selections.

REFERENCES

- Alexopoulos K, Karagiannidis A, Tsakalof P (1991). Development of macroscopic and microscopic characteristics of ejaculates from Chios, Serres and Karagouniki breed lambs. *Theriogenology*. 36(4): 667-80.
- Avellaneda Y, Rodríguez F, Grajales H, Martínez R, Vasquez R (2006). Puberty determination in rams according to body characteristics, and evaluation of quality of ejaculate and testosterone. *Livestock Research for Rural Development*. 18(10).
- Belibasaki S and Kouimtzi S (2000). Sexual activity and body and testis growth in prepubertal ram lambs of Friesland, Chios, Karagouniki and Serres dairy sheep in Greece. *Small Ruminant Research*. 37(1-2): 109-113.
- Bielli A, Pedrana G, Gastel A, Castrillejo A, Morana A, Lundeheim N, Forsberg M, Rodriguez-Martinez H (1999). Influence of grazing Management on the seasonal changes in testicular morphology in Corriedale rams. *Animal Reproduction Science*. 56: 93-105.

- Bielli A, Gastel MT, Pedrana G, Moraña A, Castrillejo A, Lundeheim N, Forsberg M, Rodriguez-Martinez H (2000). Influence of pre-and post-pubertal grazing regimes on adult testicular morphology in extensively reared Corriedale rams. *Animal Reproduction Science*. 58:73-86.
- Bielli A, Pérez R, Pedrana G, Milton JTB, Lopez A, Blackberry MA, Duncombe G, Rodriguez-Martinez H and Martin GB (2002). Low maternal nutrition during pregnancy reduces the number of Sertoli cells in the newborn lamb. *Reproduction, Fertility and Development*. 14(6): 333-337.
- Blache D, Chagas LM, Blackberry MA, Vercoe PE, Martin GM (2000). Review. Metabolic factors affecting the reproductive axis in male sheep. *Journal of Reproduction and Fertility*. 120:1-11.
- Bilgin OC, Emsen E, Davis ME (2004). Comparison of non-linear models for describing the growth of scrotal circumference in Awassi male lambs. *Small Ruminant Research*. 52 (1 -2): 155-160.
- Brown BW (1994). A review of nutritional influences on reproduction in boars, Bulls and rams. *Reproduction Nutrition and Development*. 34(2): 89-114.
- Emsen E (2005). Testicular development and body weight gain from birth to 1 year of age of Awassi and Redkaraman sheep and their reciprocal crosses. *Small Ruminant Research*. 59(1): 79-82.
- Fernández M, Giráldez FJ, Frutos P, Lavín P, Mantecón AR (2004). Effect of undegradable protein supply on testicular size, spermogram parameters and sexual behavior of mature Assaf rams. *Theriogenology*. 62(1-2): 299-310.
- Fernández M, Giráldez FJ, Frutos P, Hervás G, Mantecón AR (2005). Effect of undegradable protein concentration in the post-weaning diet on body growth and reproductive development of Assaf rams. *Theriogenology*. 63 (8): 2206-2218.
- Fourie PJ, Schwalbach LM, Naser FWC, Van der Westhuizen C (2004). Scrotal, testicular and semen characteristics of young Dorper rams managed under intensive and extensive conditions. *Small Ruminant Research*. 54:1. 53-59.
- Herrera-Alarcón J, Villagomez-Amezcuca E, González-Padilla E, Jimenez-Severiano H (2007) Stereological study of postnatal testicular development in BlackBelly sheep. *Theriogenology*. 68:582-591.
- Hötzel MJ, Markey CM, Walkden-Brown SW, Blackberry MA, Martin GB (1998). Morphometric and endocrine analyses of the effects of nutrition on the testis of mature Merino rams. *Journal of Reproduction and Fertility*. 113: 217-230.
- Latendresse JR, Warbritton AR, Jonassen H, Creasy DM (2002). Fixation of Testes and Eyes Using a Modified Davidson's Fluid: Comparison with Bouin's Fluid and Conventional Davidson's Fluid. *Toxicologic Pathology*. 30:524-533.
- Matos CAP, Thomas DL, Nash TG, Waldron DF, Stookey JM (1992). Genetic Analyses of Scrotal Circumference Size and Growth in Rambouillet Lambs. *Journal Animal Science*. 70:43-50.
- Mert H, Karakus K, Yilmaz A, Aygun T, Mert N, Apaydin B and Seyhan E (2009). Effects of Genotype on Testis, Semen Quality, and Mineral Composition of Semen in Various Ram Breeds. *Biological Trace Element Research*. 132:1-3. 93-102,
- Mukasa-Mugerwa E and Ezaz Z (1992). Relationship of testicular growth and size to age, body weight and onset of puberty in Menz ram lambs. *Theriogenology*. 38 (5):979-88.
- Murray PJ, Rowe JB, Pethick DW and Adams NR (1990). The effect of nutrition on testicular growth in the Merino ram. *Australian Journal of Agricultural Research*. 41(1) 185 - 195.
- Saab SA, Sleima FT, Nassar KH, Chemaly I, El-Skaff R (1997). Implications of high and low protein levels on puberty and sexual maturity of growing male goat kids. *Small Ruminant Research*. 25:17-22.
- Salhab SA, Zarkawi M, Wardeh, MF, Al-Masri, MR, Kassem, R (2001). Development of testicular dimensions and size, and their relationship to age, body weight and parental size in growing Awassi ram lambs. *Small Ruminant Research*. 40:187-191.
- Söderquist L and Hultén F (2006). Normal Values for the Scrotal Circumference in Rams of Gotlandic Breed. *Reproduction in Domestic Animals*. 41(1):61-62.
- Steger K, Wrobel KH (1994). Immunohistochemical demonstration of cytoskeletal proteins in the ovine testis during postnatal development. *Anatomy and Embryology*. 189:521-530.
- Ungerfeld R and Silva L (2004). Ewe effect: endocrine and testicular changes in experienced adult and inexperienced young Corriedale rams used for the ram effect. *Animal Reproduction Science*. 80:3.251-259
- Valencia MJ, Trujillo QMJ, Espinosa MMA, Arroyo LJ, Berruecos VJM (2005). Pubertad en corderos Pelibuey nacidos de ovejas con reproducción estacional o continua. *Revista Científica, FCV-LUZ*. XV(5):437-442.