Chapter

Factors Characterizing Puberty in Ram Lambs of Four Breeds Raised Under High Altitude Conditions

Harvey Lozano, Jimmy Vargas, Liliana Chacón and Nathalie Kirschvink

Abstract

The aim of this study was to compare ram lambs of four Colombian wool breeds raised under high altitude conditions to describe evolution of semen characteristics, body development, and libido and plasma testosterone. Corriedale, Hampshire, Romney Marsh, and Creole rams were enrolled since the age of 4 months for libido and testosterone (maximum, mean and amplitude) assessment, whereas semen collection was performed between 6 and 11 months of age by use of electro-ejaculation. Beside analysis of variables in function of breed and over time, a semen maturity score, considering semen volume, mass motility, individual progressive motility, concentration and % of living spermatozoa was established in function of adult rams' reference data. Colombian Creole displayed significantly higher results regarding all variables and showed the most important body development at each time point of the study.

Keywords: male lamb, non-seasonal, puberty, maturity, semen, libido, testosterone, breed

1. Introduction

Puberty corresponds to the transition into adulthood and can be defined in rams as the time when fertile spermatozoa are present in the ejaculate [1]. For some authors it is defined as the time when rams show 'interest' in females in estrus by successive mounting with ejaculation [2]. It is also described as the stage of sexual maturation when a ram can display complete sexual behaviour, to produce and to release gametes [3]. Another definition of puberty in ram lambs is based on a sustained rise in plasma testosterone concentrations over three consecutive blood samplings performed in one week and confirmed by the presence of spermatozoa in the ejaculate with at least 30% mass motility [1, 4]. In general, sexual development of ram lambs appears to be more closely associated with body growth than with chronological age. Body weight can be a good criterion for the achievement of puberty than chronological age alone [2]. Rate of testis growth is reported to be more rapid in lambs of highly prolific breeds such as the Finnish Landrace than in less prolific breed [5]. Louda et al, after observing testis growth and small but consistent differences in the development of sexual activity and

of sperm production, suggested that young rams of prolific breeds (Romanov and Finnish Landrace) may differ in their future reproductive performance. The early pubertal development associated with increased body weight is desirable in terms of improved reproductive performance [5, 6]. Broad information about the onset of puberty is of considerable importance for successful reproductive management [7]. On one hand, it is important to avoid inbreeding by separating prepubertal rams from ewes, especially in counties with extensive breeding practices and poor separation between animal groups of different ages or different reproduction status. On the other hand, insufficient sexual maturity of young rams may lead to reduced flock fertility. A few information is available in terms of testosterone levels, semen quality, libido and sexual performance at high altitude conditions in a non-seasonal country [8].

Seasonality is a main effect in the onset of rams' puberty. Animals live under the influence of seasonal fluctuations of environmental conditions with variable amplitudes frequently more marked in the higher latitudes and altitudes [9, 10]. Photoperiod is the key environmental signal timing in the reproductive cycle because the effect of season and/or day length has been studied as a main factor in onset of puberty in different breeds of young rams. Rams' sensitivity to photoperiod is different from ewes. Reproductive season can be influenced by birth date and puberty time. During spring and summer, a persistent hyper prolactinaemia was associated with low circulating FSH concentrations in Texel rams [11]. Young rams may reach puberty by 4 months of age during the first fall season, while the onset of puberty may be delayed until 9–12 months for rams born late in the lambing season and if additionally, the situation is accompanied of poor feeding and poor climatic conditions [12, 13]. In Karakul rams in southern Iran is observed how lambs born at the beginning of spring is sexually more precocious than lambs born later [14].

It is well known that interactions between body and testis growth, sperm production and testosterone secretion is complex since early pre-pubertal ages and, in many aspects, such events can be influenced by both the genetic background of animals and the environment where they are raised [2]. Plasma FSH concentrations were lower in Texel rams than in Suffolk and Ile-de-France rams during both pre-pubertal and pubertal periods [11]. Emsen [5] showed that crossing Awassi sheep with a native breed Redkaraman with relatively higher growth rate can considerably improve the early pubertal development of rams. Puberty of Awassi ram lambs in improved flocks, started around seven months of age and at on average weight of 34.6 kg [15]. In general, sexual development of ram lamb appears to be more closely associated with body growth than with chronological age and the live weight of the ram lambs at puberty is probably related to the genotype [2]. In that research ram lambs of breeds Friesland and Chios attained puberty when they reached approximately 50% of their adult body weight. By comparing different breeds and hormone levels during pre and pubertal time it was shown that Canadian ram lambs had significantly lower FSH levels than other breeds like Finnish Landrace. However, as adults the same group of Canadian rams had larger testicles and better semen quality [12]. In pubertal development, scrotal circumference was highly and positively correlated with live weight, but negatively correlated with inhibin and FSH concentrations in rams of Suffolk and DLS (Dorset × Leicester × Suffolk) [13]. Rate of testis growth was more rapid in lambs of Finnish Landrace with high prolificacy than a less prolific breed such as Merino [16].

Onset of puberty time in ram lambs is considerably influenced by nutrition. Prepubertal nutritional restrictions delay testicular growth and the rate of sexual development in ram lambs is highly dependent on energy intake and live weight gain [17].

Nutritional restrictions can influence the activity of the hypothalamus-pituitary axis and thus reduce gonadotropin levels in ram lambs. It can even be influenced during prenatal period based on mother's nutrition during pregnancy reducing lamb's pituitary capacity to liberate gonadotropins like LH [17]. The scrotal circumference of rams under an intensive feeding management increased by 4.4%, while decreased for rams under extensive conditions by 2% due to poor nutritional conditions [18].

The social environment in which male lambs are reared appears to influence some aspects of reproductive behavior: the exposure to cyclical ewes during the first 6 months of life is fundamental to induce an increase in testosterone concentrations and in testicular size and also the social rank of male lambs during pre-pubertal development affected reproductive performance of adult rams [19].

Sexual performance of rams having previously direct contact with females at 7–9 months of age was enhanced in comparison with rams without that previous experience [20]. In a study developed to evaluate the possible influence of litter size on the onset of puberty and hormone levels it could be realized how lambs born as singletons had lower testosterone levels at 8 months of age than those born as twin or triplets [12]. Ungerfeld and González-Pensado [21] reported a study in which intensive male-male sexual behavior is described in pubertal lambs with approximately the same age (less than 6 months) and it was shown how more dominant lambs displayed more intensive sexual behavior toward subordinate males and cyclical ewes, whereas more subordinate males received more mounts from other males and were less active to mount. Ram lambs of the breed Polish Milk Sheep, which is more prolific and attains puberty earlier, were more active in sexual play than ram lambs of the Polish White-headed Mutton [22].

2. Materials and methods

2.1 Animals and location

The study was conducted between July 2012 and April 2013 at the experimental farm of the National University of Colombia at 2650 m of altitude at 4°42′ latitude north and 74°12′ longitude west, near Bogotá (Colombia).

Twenty-four ram lambs, aged 3–4 months at the beginning of the trial and from four different breeds (6 per breed) were enrolled in the study. Animals belonged to the native Colombian Creole and three foreign adapted breeds that are frequently used by Colombian breeders: Hampshire, Corriedale and Romney Marsh. The animals were born in the center and were selected of base of a selection index. They were grazing all the time of the experiment and each animal received daily 200 g of a pelleted concentrate mixture and 300 g of trefoil hay. Mineral salts and water were provided ad libitum.

The protocol was approved by the Bioethics committee of the Faculty of Veterinary Medicine and Animal Science of the National University of Colombia (Acta: CD-071-2014).

2.2 Assessment of semen characteristics in ram lambs

Once per month, ram lambs were collected by electro ejaculation, penis was exposed from the prepuce cavity and the urethral process was gently introduced into a conical tube previously to start ejaculation process. Semen was placed in a water bath

at 37°C and subjected to the following tests: (1) Volume was measured in a conical glass tube graduated with 0.1 mL optically visible intervals; (2) motility (Mass: semen was assessed for semen wave motion graded on a subjective scale ranging from 1 to 5, where 1 was scored when there was no mass movement and 5 represented vigorous waves of sperm motion and Individual progressive motility %).

Semen concentration was determined using a standard spectrophotometer (540 nm). (4) The proportion of live and dead spermatozoa was determined using the nigrosine-eosin staining technique by counting at least 200 spermatozoa under oil immersion objective (1000×) random fields. The proportion of morphologically abnormal spermatozoa was also determined by examining 200 spermatozoa in an eosin-nigrosine smear under the same magnification. Abnormal spermatozoa were then classified into proportion of spermatozoa with head abnormalities, midpiece abnormalities, tail abnormalities, proximal droplet, distal droplet, detached heads or tailless spermatozoa [23]. All examinations were performed by the same operator.

2.3 Plasma testosterone concentration

Blood samples were collected every month during 5 hours at 30 minutes intervals using heparinized venoject® tubes and centrifuged (1500 × g for 15 min). The plasma was recovered and stored at −70°C. The concentration of testosterone in the plasma samples was measured in duplicate by an adapted enzyme immunoassay using a diagnostic commercial kit (DS-EIASteroid®-Testosterone RH-353). A calibration curve was used in order assess concentrations ranging from 1.25 to 40 nmol/l of testosterone in plasma. Known samples of a mature adult ram and of an ovariectomized ewe were used as positive and negative control respectively on each plate. Lower detection limit was 0.2 nmol/l and inter-and intra-assay were 6.7 and 5.5%, respectively. In each ram, testosterone curves were plotted over each 5 h period to determine the maximum value. The difference between recorded maximum and minimum concentration allowed to calculate testosterone amplitude.

2.4 Body measurements

Body weight was assessed once monthly using an electronic balance. Scrotal circumferences using a scrotal tape was evaluated also monthly.

3. Statistical analysis

Semen characteristics, testosterone concentration (peaks, amplitude, mean) were analyzed using a generalized linear model (GLM) for repeated measures analysis. The model contained effects due to breed and period. Mean differences in body weight (kg), scrotal circumference (cm) and scrotal circumference/body weight was compared by Duncan's method. The level of significance was set at $P < 0.05$ for all tests. Data was analyzed using SAS System (SAS version 9.12, SAS Institute Inc.). A semen maturity score, aiming at comparing the global semen quality of young rams in function of semen quality of adult rams of the same breed, was established as follows: Semen characteristics of five adult rams of each breed collected over a period of one year was averaged and considered as reference value. These breed-specific reference values were established for semen volume, concentration, mass motility (MM), individual progressive motility (IPM) and percentage of living spermatozoa. At each

month of collection, semen variables of the ram lambs were expressed as % in function of the adult rams' reference value. This percentage was classified in function of three semen maturity levels: when 50, 75 or 100% of the adults' value were reached, a note of 1 was accorded to this semen variable. If the % was lower than 50, 75 or 100%, a note of 0 was attributed. At each month of sampling, the maturity score establishing the sum of the notes attributed for semen volume, concentration, MM, IPM and % of living spermatozoa for achievement for 50, 75 or 100% of adult rams' semen quality was calculated.

4. Results

Table 1 shows semen characteristics and body measurements in function of breed and indicates significant time-, breed- or interaction effects. No breed effect was found for semen volume, whereas Corriedale rams showed lowest values for concentration, MM, IPM ad % of normal spermatozoa ($p < 0.05$). **Figure 1** displays detailed evolution of these variables (volume, MM, IPM, concentration and % of normal spermatozoa) over time and in function of breed. All variables achieved a stable level when the rams were 10 and 11 months old. Creole rams showed consistently higher values for all variables except semen volume, whereas Corriedale rams generally showed the lowest values.

Table 2 shows the evolution of the semen maturity score per breed and over time. Although no significant differences between breeds were found, a clear trend for more rapid development were found in Creole and Romney Marsh ram lambs.

Table 3 shows as the evolution of body development (expressed in function of adults' ram weight) over time. Creole and Romney Marsh display highest values at begin of the investigation whereas Creole rams maintained this rapid development until the age of 11 months.

Figure 2a and **b** display maximum testosterone concentration and testosterone amplitude in function of breed and over time. Creole ram lambs had significantly higher values. The timerelated increase was mostly observable in all breeds between 3 and 6 months of age. There were not correlations between testosterone levels and semen quality, sexual activity, or body measurements. In general, the first expressed signs of interest in the oestrous females occurred prior to the sustained rise in testosterone.

5. Discussion

This study aimed at describing semen characteristics, body development, and libido and plasma testosterone concentration in young rams of four economically important wool breeds in high altitude conditions in Colombia. Prior considering and discussing results in detail some drawbacks must be mentioned. Semen collection was performed by electro-ejaculation since the age of 6 months and as expected and described by others [1, 18], semen quality and testosterone levels recorded at this age suggested that puberty was already achieved by most of the rams. The relatively late start of semen collection was due to the fact that despite regular training of the rams, semen collection by use of an ewe in estrus was not successful in all rams, which meant that semen quality data were only available in a few rams. Therefore, the investigators decided to switch to semen collection by use of electro-ejaculation. Even

	n	Creole	Romney M	Hampshire	Corriedale	Effect breed	Effect collect	Effect breed * collect
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD			
		7	7	7	7			
Semen character								
Volume (ml)	6	1.10 ± 0.3	1.10 ± 0.38	1.30 ± 0.4	1.20 ± 0.39	NS	$P < 0.05$	NS
Conc. (millions/ml)	6	2431.4 ± 972.3[a]	2512.4 ± 734.9[a]	2494.7 ± 1021.6[a]	2016.6 ± 1013.9[a]	$P < 0.05$	$P < 0.05$	NS
Mass motility (0–5)	6	4.10 ± 0.6[a]	3.90 ± 0.99[a]	3.8 ± 0.85[a]	3.20 ± 1.37[b]	$P < 0.05$	$P < 0.05$	NS
IPM (%)	6	85.1 ± 7.4[a]	80.9 ± 14.2[ab]	79.4 ± 12.5[b]	72.8 ± 16.54[c]	$P < 0.05$	$P < 0.05$	NS
N (%)	6	85.4 ± 2.9[a]	85.3 ± 3.12[a]	85.1 ± 2.72[a]	80.8 ± 6.24[b]	$P < 0.05$	$P < 0.05$	NS
Body measure								
Scrotal circumference (cm)	6	28.4 ± 2.2[a]	32.3 ± 1.54[b]	31.3 ± 2.13[c]	29.1 ± 2.37[a]	$P < 0.05$	$P < 0.05$	NS
Body weight (Kg)	6	34.9 ± 5.7[a]	44.5 ± 5.58[b]	39.7 ± 6.69[c]	39.1 ± 6.83[c]	$P < 0.05$	$P < 0.05$	NS
SC/BW	6	0.82 ± 0.09[a]	0.74 ± 0.07[b]	0.80 ± 0.11[a]	0.76 ± 0.08[b]	$P < 0.05$	$P < 0.05$	NS

Values with the same letter are not significantly different, $p < 0.05$.
NS, not significantly different.

Table 1.
Mean (±SD) seminal characteristics and body measure evaluated in ram lambs in four breed (Creole, Romney, Corriedale and Hampshire).

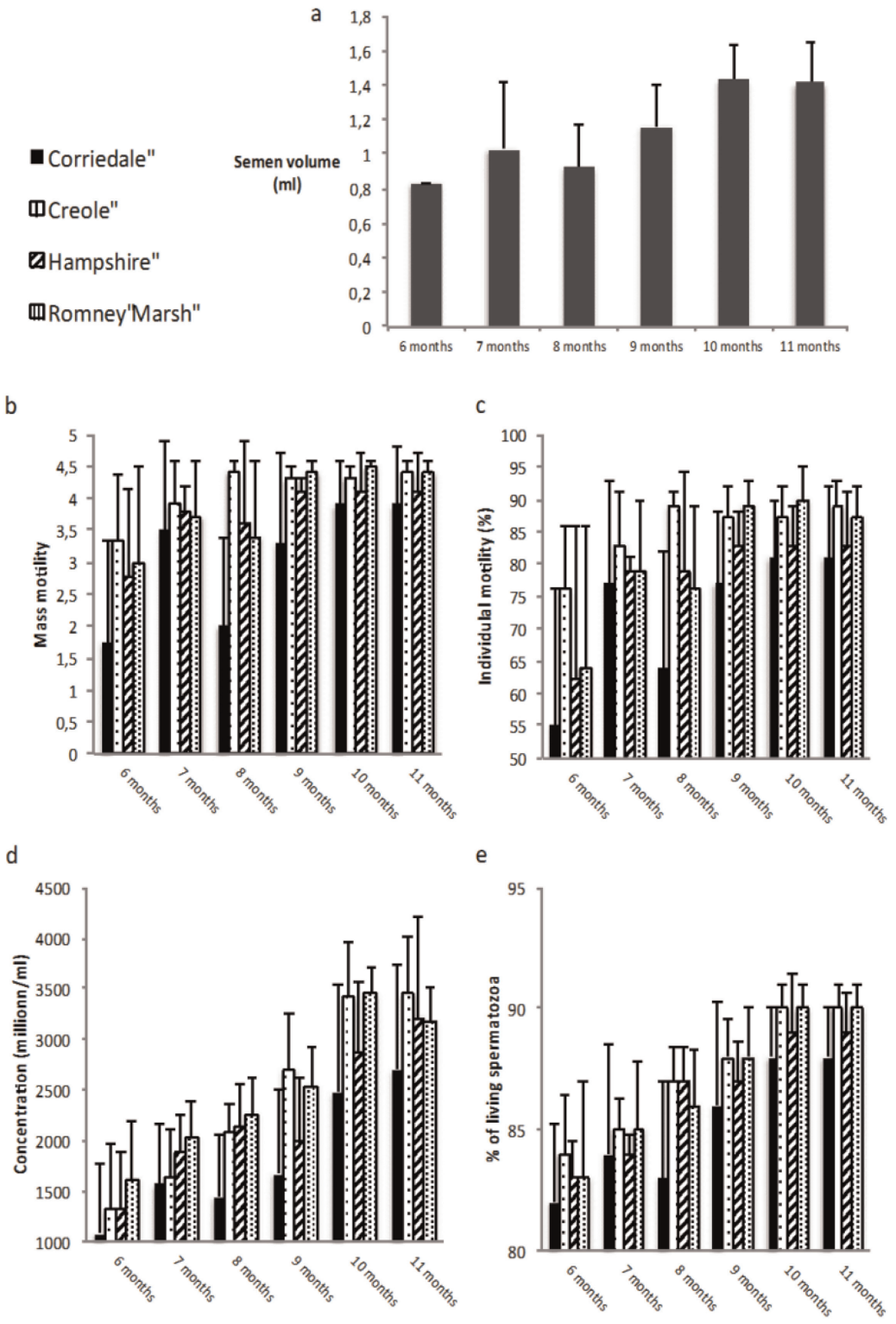

Figure 1.
Evolution of semen quality in rams of four breeds (7 animals/breed) over time in rams aged between 6 and 11 months. A significant time effect was found for semen volume (a), whereas significant time and breed effects were found for mass motility (b), individual progressive motility (c), concentration (d) and % of living spermatozoa (e). Data are shown as mean and standard deviation.

Age	Number of rams with a maturity score > 50%				Number of rams with a maturity score > 75%				Number of rams with a maturity score > 100%			
Months	**CO (n = 7)**	**CR (n = 7)**	**HA (n = 7)**	**RM (n = 7)**	**CO (n = 7)**	**CR (n = 7)**	**HA (n = 7)**	**RM (n = 7)**	**CO (n = 7)**	**CR (n = 7)**	**HA (n = 7)**	**RM (n = 7)**
6	1	1	0	1	0	0	0	0	0	0	0	0
7	2	2	3	4	0	0	0	0	0	0	0	0
8	3	4	3	6	0	0	0	0	0	0	0	0
9	4	7	5	7	0	4	0	0	0	0	0	0
10	4	7	6	7	2	5	4	7	0	2	0	0
11	5	7	6	7	4	6	6	7	0	2	0	0
Total (n) and % of observations fulfilling maturity	19	28	23	32	6	15	10	14	0	4	0	0
	45%	67%	55%	76%	14%	36%	24%	33%	0%	10%	0%	0%

Maturity scores consider semen quality corresponding in terms of volume, concentration, mass motility, individual progressive motility and % of normal spermatozoa when 50, 75 or 100% of adults' semen quality is achieved. Data per line are shown as number of rams whose semen corresponds to the fixed maturity score.
CO, Corriedale; CR, Creole; HA, Hampshire; RM, Romney Marsh.

Table 2.
Semen maturity scores of Corriedale, Creole, Hampshire and Romney Marsh ram lambs tested between 6 and 11 months of age.

Age expressed in months	CO (n = 7)	CR (n = 7)	HP (n = 7)	RM (n = 7)	Breed effect
6	47.5 ± 8.4	56.5 ± 5.0	46.8 ± 4.3	51.0 ± 4.3	$P < 0.05$
7	48.3 ± 7.9	58.2 ± 4.6	47.7 ± 3.7	51.6 ± 4.0	$P < 0.05$
8	50.3 ± 7.5	67.7 ± 5.8	52.2 ± 5.8	53.6 ± 4.5	$P < 0.05$
9	54.7 ± 7.3	75.1 ± 7.9	57.8 ± 7.5	57.2 ± 5.3	$P < 0.05$
10	55.9 ± 8.1	79.4 ± 8.5	60.6 ± 11.8	62.0 ± 5.9	$P < 0.05$
11	56.4 ± 11.8	82.1 ± 8.6	65.4 ± 9.7	64.6 ± 4.9	$P < 0.05$

Data are expressed as % in functions of adult rams' body weight of the same breed. Means and SD are shown for each breed.
CO, Corriedale; CR, Creole; HA, Hampshire; RM, Romney Marsh.

Table 3.
Evolution of body development in function of breed and over time.

if the semen characteristics do not importantly change when natural versus electric ejaculation are compared this methodological difference must be kept in mind when ram lamb semen data are expressed in function of adults' semen data, leading to an underestimation of young rams' semen quality. Although the semen quality score allowed to consider simultaneously all important semen characteristics (volume, MM, IPM, concentration and % of living spermatozoa) and thereby allowed to assess the ability of young rams at the end of the puberty (rather than to consider each variable separately), it remains a matter of fact that onset of puberty occurred before or just around first semen collection and that a precise age of onset of puberty could not be established for each ram and/or each breed. Regarding the end of the study, it appears that albeit a stabilisation since at least two months of all parameters (except semen concentration), it is impossible to define time point sexual maturity. As evidenced by the semen quality score, only two Creole rams showed semen characteristics that perfectly corresponded to those of adults of the same breed. The study should have been prolonged to assess when semen maturity was reached. Nevertheless, the present study describes that Colombian Creole appears as the most precocious breed: indeed, semen variables, semen maturity score, behavioural aspects as well as plasma testosterone levels were significantly increased in comparison to the other breeds, especially to Corriedale rams whose performances were almost always lower than in the other breeds. At the same time, it became apparent that development was more important in Creole rams whose relative body weight equalled 55–56% at the age of 6 months (versus 47–51% in the other breeds) and 80–85% at the age of 11 months (versus 55–65% in the other breeds). Moreover, when considering semen quality data (**Figure 1**), it can be said that since the age of 8 months almost all rams, except some Corriedale rams whose semen concentration remained below 2000 million spermatozoa/ml, were able to ensure reproduction. Indeed, the only limiting factor in terms of reproductive capacity seemed to be concentration.

It is interesting to state that this local breed appears to be optimally adapted to high altitude Andes conditions, whereas the foreign breeds, although imported since more than 50 years, show significant differences. They mostly account for a larger body size, meaning that the minimal % of body development is achieved later point, but seem also to depend on other factors. Indeed, testosterone levels were significantly higher in Creole rams and might impact semen quality. Another aspect was evaluated,

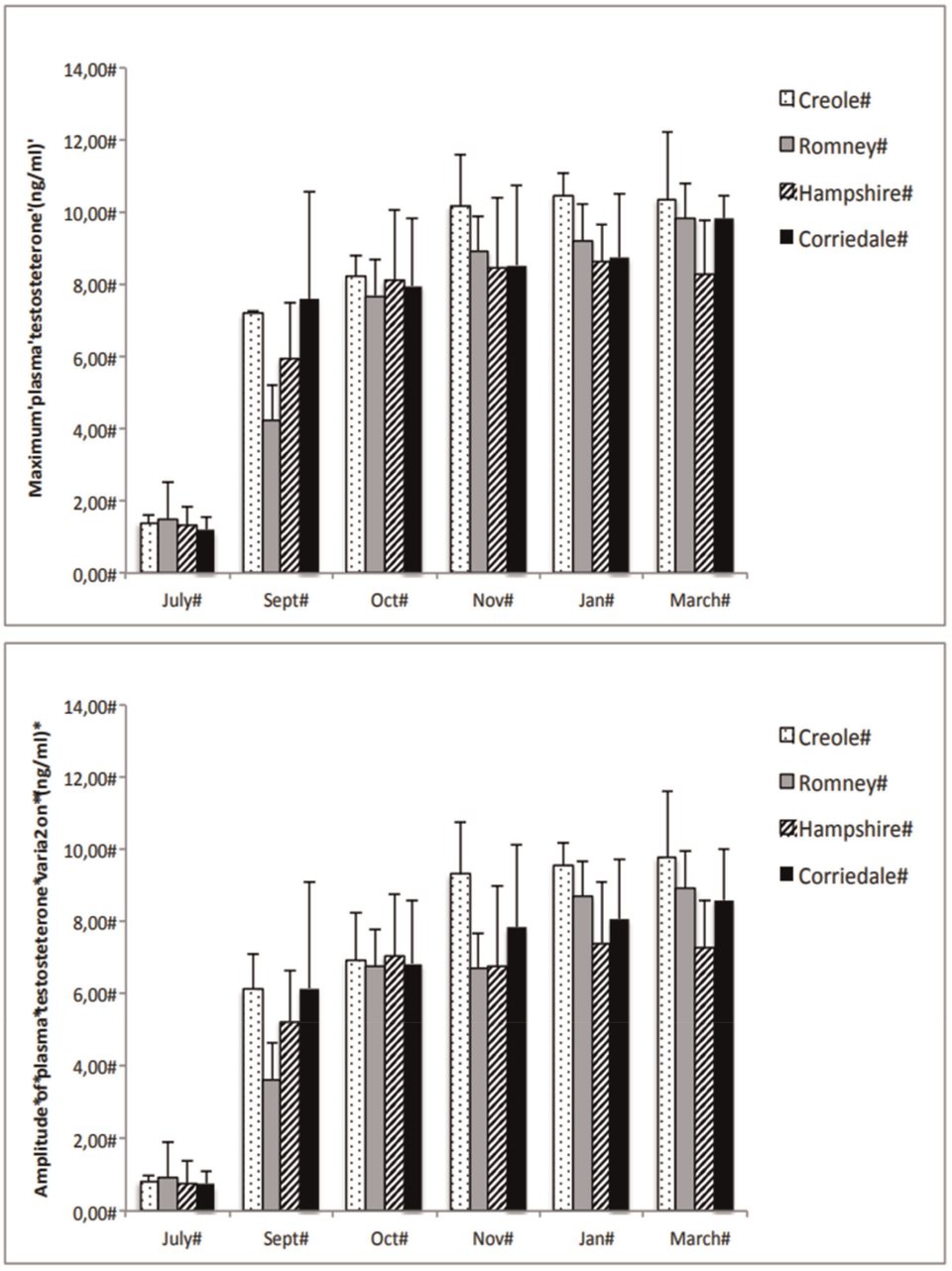

Figure 2.
(a) Maximum plasma testosterone concentrations assessed between the age of 3 (July data) and 10 (March data) months in Creole, Romney Marsh, Hampshire, and Corriedale ram lambs. Data are shown as means with SD. A significant collection effect was recorded over time, as well a significant breed effect with Creole rams showing significantly higher testosterone levels, $p < 0.05$. (b) Plasma testosterone amplitude assessed between the age of 3 (July data) and 10 (March data) months in Creole, Romney Marsh, Hampshire, and Corriedale ram lambs. Data are shown as means with SD. A significant collection effect was recorded over time, as well a significant breed effect with Creole rams showing significantly higher testosterone levels, $p < 0.05$.

but results are not presented here, it is about sexual behavior of the ram lambs since they were three until one year of age.

In Colombia some time after this research was developed, this group could perform a trial under low altitude conditions and ram lambs belonged to hair breeds instead of wool, finding some results in concordance to these results, especially about testosterone levels during ram lambs growing up. Into that hair groups experiment, time of evaluation was shorter than this research, but some parameters could be compared and the new information was useful for the good understanding of puberty

in ram lambs in a country as Colombia where there is different altitude in farms despite of being a non-seasonal country [24].

In conclusion, this investigation describes how semen quality, libido and plasma testosterone evolve over time an in four Colombian wool breeds and allows to point out the importance of body development to achieve satisfying reproductive abilities. It was shown that almost all variables were improved in Colombian Creoles, whereas Corriedale showed the lowest development.

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